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# 132. THE NUTRITIVE VALUE OF PROTEINS FOR MILK PRODUCTION

## IV. A COMPARISON OF THE PROTEINS OF (*a*) SPRING AND AUTUMN GRASS, (*b*) GRASS CONSERVED AS SILAGE (A.I.V. ACID TREATED, MOLASSES TREATED AND ORDINARY UNTREATED), AND (*c*) GRASS CONSERVED BY DRYING, WITH NOTES ON (i) THE EFFECT OF HEAT TREATMENT ON THE NUTRITIVE VALUE AND (ii) THE SUPPLEMENTARY RELATIONS OF FOOD PROTEINS

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(With 10 Figures)

IN previous papers (1, 2) it was shown that the deficiency in any food protein of an essential amino acid, such as lysine or tryptophane, resulted in a relatively poor utilization of the protein for milk production. This conclusion was based on a series of experiments with cows using food proteins of high and low lysine and tryptophane content. The results in the present paper deal mainly with the proteins of young grass in the fresh state and also conserved as silage or by drying.

The importance of the subject cannot be denied since, in view of results at various centres in which yields of $3\frac{1}{2}$–$5\frac{1}{2}$ tons of dry matter per acre were obtained by the conservation of young grass, a vast store of a high protein concentrate could be obtained for winter feeding of live stock. It has been shown by various workers (3, 4, 5, 6) that the feeding of grass, either fresh or conserved, gives a higher content of both vitamin A and carotene in the milk secreted. Various feeding trials have also been carried out (7, 8, 9, 10) to determine the nutritive value of the foodstuffs as a whole, but unfortunately, from the point of view of protein value, the level of intake was too high. As regards the biological value of the proteins of young grass, Crampton (11) has recently published results in which feeding trials were conducted using young growing rabbits as experimental animals. He found that marked differences occurred between manured and unmanured grass, the former having a higher nutritive value. Even with heavy manuring, however, the grass was deficient in cystine. With ruminants this deficiency will not affect the nutritive value of the proteins, unless, possibly, only minimal amounts of cystine are present (12).

The present paper is divided into three sections, the first dealing with fresh and conserved grass, and the last two with the effect of heat treatment and the supplementary value of proteins. For ease in discussion and reading, it has been decided to put all the tables and figures as an appendix.

## Experimental methods

### (*a*) *Animals*

Pedigree Ayrshire cows, in the third or fourth lactation and from 4 to 6 weeks calved, were used. The weights of the animals were about 1000 lb.

### (*b*) *Rations*

The actual rations fed are shown in Tables I–IV. Throughout each experiment the cows received a maintenance ration of straw, oats and beet pulp, at the rate of 0·6 lb. protein equivalent and 6 lb. starch equivalent per 1000 lb. live weight. The production rations were fed at the rate of 2·5 lb. starch equivalent and 0·44 lb. protein equivalent per 10 lb. milk. The concentrates under investigation were fed in the production ration in quantities which supplied equivalent amounts of nitrogen to the ration, the rations being balanced with oats and maize. Water was allowed *ad lib.*

The blood-meal ration consisted of a mixture of blood, Paisley meal and maxtrex. The low-temperature dried material was obtained from Messrs W. and J. Dunlop, Ltd., of Dumfries, and the high-temperature blood meal was the product obtained in the open market. The special blood meal mixtures referred to in Table II were made by Messrs W. MacKean, Ltd., of Paisley.

The fresh grass, silage and dried grass were obtained from a mixed pasture, well manured with nitrogen, phosphorus and potassium. Where a comparison was made between young spring (May) and autumn (October) grass in the same year the same field was used and kept manured and cut until the autumn feeding period was completed. The three types of silage were made over a period of less than 3 days with young grass cut in the first week of August. The dried grass was made from young pasture grass and dried in a Ransome drier.[1] In all cases where a comparison was made between fresh and conserved grass the same field of mixed herbage was used.

Analyses of the fresh grass, dried grass and the three types of ensilage showed that the protein content at the time of feeding was about 18 per cent. of the dry matter, except for the autumn grass which contained about 20–23 per cent.

Although in the making of the A.I.V. (acid-treated) silage the amount of acid to be added was controlled by *p*H determinations, it was found that when the silage pits were opened the ensiled grass had a *p*H ranging from 1·7 to 3·0. Due to the high acidity, cow 4 refused the silage after 2 days, although part of the acidity was neutralized to *p*H 4·0 with sodium bicarbonate.

[1] The air temperatures in the two sections of this drier are 300 and 220° F. respectively.

As regards the general health of the animals it must be noted that only with cows 3, 4 and 5 on the silage periods were any ill effects noted. The most marked effect after the silage feeding was a loss of "bloom" and a general dullness of the animals, a state closely approaching one of lethargy.

## I. Discussion of results of fresh and conserved grass

### (*a*) *Milk yield*

In the previous papers (1, 2) it was shown that the feeding of a deficient protein, at the low level of 0·44 lb. protein equivalent per 10 lb. milk, ultimately led to a reduction in milk yield. Reference to Fig. 1 shows that certain of the feeds cause a lowering of milk yield. The dotted lines show the expected lactation curve for an animal fed throughout the experiment on an adequate diet. From the first two curves it is clear that fresh spring grass shows no fall in milk when compared with low-temperature dried blood meal, a food known to be efficient in all respects (2). On the other hand, in the following three curves young autumn grass shows a marked reduction in milk yield in comparison with the blood meal. From a comparison of these and of the remaining curves it is obvious that of the grass rations only those containing autumn grass, fresh or dried, caused a definite fall in the milk yield, while rations containing fresh or dried spring grass or either of the three types of silage gave the expected maximum yield. The results with cow 6 indicate that spring grass is a better source of protein for milk production than bean meal.

The relatively prolonged period between the change in ration and the fall from, or return to, the normal lactation curve probably represents the utilization or formation of reserve N in the body. A similar "lag" in the effect of different rations on milk yield has been observed in our earlier work.

### (*b*) *Total nitrogen of milk and urine*

Figs. 2–10 show the relationship between the total output of N in the milk and urine during the experimental periods. In interpreting the results allowance must be made for the gradual increase in urinary N resulting from the normal decrease in milk yield during the lactation period, since the quantity of protein fed throughout the experiments was constant, being based on the initial milk yield.

The results show clearly that a relatively high wastage accompanies the feeding of autumn grass, fresh and dried, and of bean meal, whereas the wastage is markedly lower with the spring grass, fresh and dried, and the silage.

### (*c*) *Nitrogen partition in the urine*

It will be seen from Tables V–XII that the feeding of autumn grass, fresh or dried, and beans results in an increase in the excretion of exogenous metabolites, through deamination of the excess circulating amino acids. As regards N partition, the results are not sufficiently consistent to warrant any generalization, though there is some indication that the percentage of ammonia N is

higher with the autumn grass and beans. There are no significant variations in the output of amino-acid N during the series of experiments.

The A.I.V. silage caused a marked increase in urinary ammonia excretion, whereas with the molasses and ordinary silage the increase was very slight. This finding confirms the work of Peterson *et al.*(6). These authors found a very large increase in urinary ammonia on feeding A.I.V. silage to cows, together with a lowering of the *p*H and fixed $CO_2$ content of the urine. Since no increase above normal was noted in the total urinary N output it seems safe to conclude that the cause of the increased ammonia excretion is the ingestion of the acid silage.

With regard to endogenous metabolism, the variations in the output of creatine are marked. With fresh autumn grass the increase in creatine excretion is large, whereas with the dried product the increase is only slight. It is evident, comparing the total urinary N excretion and the creatine output, that there is a marked parallelism. In previous papers(1, 2, 12) it was stated that this increased creatine excretion may be due either to a catabolism of tissue N by the animal in an attempt to maintain milk production on the poor-quality proteins, or to an acidosis produced by the intensified deamination. Recently Hutchinson & Morris(13) have shown that an increase in deamination has no effect on the creatine excretion. It can only be concluded therefore that the increases noted in the present and previous experiments are the results of tissue catabolism. It is interesting to note that the fresh autumn grass causes a more marked tissue catabolism than the dried material.

With the three types of silage, on the other hand, a different state exists. The increased excretion of creatine, most marked with the A.I.V. silage, is determined solely by the acidosis produced by the ingestion of acid, and varies directly with the amount of acid ingested. That an acidosis can produce an accelerated endogenous metabolism has long been known(14).

### (*d*) *Sulphur excretion in the urine*

Tables XIII–XVI show the results of the S and N determinations in the urine. As previously noted(2, 12) the S : N ratio of the urine is greater than that of the ration fed. This is due to the fact that the S : N ratio of the milk secreted is very much less than that of the food ingested. Little evidence as to the nature of the catabolism occurring in the body can be obtained from the S : N ratio of the urine. If, however, during any feeding period an excess catabolism occurs, the nature and origin may be determined from a study of the excess N and S excreted. Since the ratio of S : N in body tissue (1 : 15·7) is very much lower than that of the food ingested it should be possible to determine whether any excess excretion in the urine is due to tissue catabolism or to excessive deamination.

It has been shown that during the feeding of fresh autumn grass a marked increase in urinary N was found, and this is accompanied by an increased excretion of S. Calculating the ratio of the excess S : excess N it is seen that

this approximates that of body tissue. With dried autumn grass, on the other hand, there is a marked excess of S but only a small excess of N, with a S : N ratio in no way approximating that of body tissue. Similar results are obtained with cow 3 on A.I.V. silage. It would appear that with the dried grass and silage the tendency for tissue catabolism was so slight that only the S-containing portion of the protein molecule was affected. This would be in complete agreement with the work of Wilson (15) and Hutchinson & Morris (13), who found that in tissue catabolism the sulphur is split off prior to the nitrogen.

The results confirm the conclusions reached in previous sections of this paper that during the feeding of fresh autumn grass there is a marked catabolism of body tissue. With the dried grass the catabolism is very much less intense and is similar to that found when an acidosis is produced by feeding on acid foodstuff such as A.I.V. silage.

### (*e*) *Nitrogen balance*

In Tables XVII–XX are shown the results of the N balance. Fairly large differences are noted, the positive and negative balances being at times remarkably high. This may possibly be the effect of relatively short experimental periods. Over the complete experimental period with each animal, except with cow 9, the balance is positive, the animals tending to retain part of the N ingested. It is peculiar, in view of the marked positive N retentions with cows 3, 4 and 5 during the periods of silage feeding, that there was such a decided loss of "bloom" and condition.

### (*f*) *Faecal nitrogen*

The present series of experiments confirm the results, previously noted (1, 2), that when a deficient protein is fed the apparent digestibility of the total ration is higher than when an adequate protein is ingested (Tables XXI–XXIV). This subject has been studied and discussed in very much greater detail elsewhere (13).

### (*g*) *Calculation of biological values*

The formula used in the calculation of the biological values was that noted in the previous papers (1, 2), i.e.

$$\text{Biological value} = \frac{X}{I_{(\text{corr.})} - (F_t - M_t) + (F_m - M_m) + (F_x - M_x)} \times 100,$$

where $X$ = total milk N; $I_{(\text{corr.})}$ = production N intake corrected for milk yield; $F_t$ = total faecal N; $F_m$ = maintenance faecal N; $F_x$ = faecal N corrected for production intake; $M_t$ = total metabolic N; $M_m$ = maintenance metabolic N; $M_x$ = metabolic N corrected for production intake.

It must be emphasized that the results (Tables XXV–XXVIII) refer, not to pure foods, but to these foods supplemented by oats and maize. Account must also be taken of the relatively high maintenance intake, and to the possible supplementary relationship between the proteins of the maintenance ration and of the production ration.

The following figures, abstracted from Tables XXV–XXVIII, show the relatively close agreement between the biological values obtained with the nine animals:

| Ration | Cow 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Blood meal | 75·1 | 80·3 | 75·9 | 84·9 | 77·8 | — | — | — | — | 79·6 |
| | 80·6 | 84·5 | | | | | | | | |
| | 80·1 | 76·8 | | | | | | | | |
| Fresh spring grass | 81·7 | 83·4 | — | — | — | 84·9 | — | — | — | 83·3 |
| Dried spring grass | — | — | — | — | — | 86·7 | 72·6 | 75·4 | 70·8 | 74·4 |
| | | | | | | | 68·1 | 75·6 | 70·1 | |
| | | | | | | | 73·0 | 77·1 | | |
| Fresh autumn grass | — | — | 64·9 | 67·7 | 60·8 | — | 52·2 | 62·0 | — | 61·5 |
| Dried autumn grass | — | — | — | — | — | — | 61·5 | 64·5 | 55·6 | 60·5 |
| A.I.V. silage | — | — | 77·2 | — | 84·2 | — | — | — | — | 80·7 |
| Molasses silage | — | — | 82·0 | 76·3 | 79·4 | — | — | — | — | 79·2 |
| Ordinary silage | — | — | 78·1 | 88·5 | 78·5 | — | — | — | — | 81·7 |
| Bean meal | — | — | — | — | — | 67·5 | — | — | — | 68·5 |
| | | | | | | 69·4 | | | | |

Too much stress must not be laid on the significance of the mean figures. The biological values not only show occasional irregularities, but differ somewhat from cow to cow. For example, all the values obtained with cows 7 and 9 appear to be low, while those with cow 6 are probably high. The errors involved through individual variations between experimental animals might have been largely overcome if it had been possible to include a common ration (say blood meal) in each experiment. This was impracticable. An attempt has, however, been made to recalculate the biological values on the assumption that the value for blood meal is 75—a figure approximating that found in earlier work. Where blood meal was not fed to an animal the biological values have been calculated by comparison with a ration previously standardized against blood meal. Thus blood meal has been used for calculating the values for cows 1–5, the equivalent value of autumn grass for cows 7–9, and the equivalent value of dried spring grass for cow 6. The resulting biological values work out as follows:

| | |
|---|---|
| Blood meal (arbitrary value) ... ... ... ... | 75·0 |
| Fresh spring grass (79, 78)... ... ... ... ... | 78·5 |
| Dried spring grass (80, 76, 73, 77) ... ... ... | 76·5 |
| Fresh autumn grass (64, 60, 59, 56, 60) ... ... ... | 60·0 |
| Dried autumn grass (66, 62, 61) ... ... ... ... | 63·0 |
| A.I.V. silage (76, 81) ... ... ... ... ... | 78·5 |
| Molasses silage (81, 67, 77) ... ... ... ... | 75·0 |
| Ordinary silage (77, 78, 76) ... ... ... ... | 77·0 |
| Bean meal ... ... ... ... ... ... ... | 63·0 |

It will be seen that the means of these values show less variations than the means of the uncorrected values. In particular the marked difference between the uncorrected means of fresh and of dried spring grass practically disappears, a result which is in agreement with the observations on cow 6, the only animal to which both types of spring grass were fed. The most striking differences in biological value, which are apparent from both the uncorrected and the corrected figures, are those between spring and autumn grass, the biological value of the former approximating that of blood meal,

and of the latter that of bean meal. There are no significant differences between the biological values of the three types of silage, all of which were made from summer grass.

## II. The effect of high temperature on the nutritive value of the proteins of blood meal

The effect of heat on the amino-acid structure of proteins has recently received much attention. Greaves & Morgan (15) noted a deterioration in the nutritive value of heated casein which could be compensated by the addition of lysine to the diet. The authors claimed that the lysine was destroyed by the heat. Block *et al.* (16), however, analysed the heated and unheated casein and found that the chemically determined lysine was not affected by heat treatment. They state that "it is not unlikely that a molecular rearrangement is brought about so that a part of the lysine precursors become resistant to enzymatic degradation. This resistance to enzymatic hydrolysis would explain in part the lowered nutritive value of the heated casein". This effect of heat treatment on the nutritive value of proteins has been amply confirmed on small animals by Chick *et al.* (19) and Morgan *et al.* (17, 18).

The importance of the subject with reference to ruminant nutrition cannot be too strongly stressed, especially with regard to the drying by heat of such valuable protein concentrates as blood meal, grass and fish meal. During the course of the experiments previously recorded on the nutritive value of fresh grass it was found possible to include with two cows periods on high-temperature dried blood meal in order to compare the product with the low-temperature dried material.

It is obvious from the first two curves in Fig. 1 that the high-temperature dried material has a marked effect on milk yield, reducing the output considerably. With the N output of urine and milk (Figs. 2 and 3) there is a largely increased excretion of urinary N, while the milk N falls markedly. From the results of the N metabolism (Tables V, VI, XIII, XVII, XXI) it is obvious that the high-temperature dried blood-meal protein is of much poorer quality than the low-temperature material. Thus the percentage urea decreases while the ammonia increases markedly; the creatine output increases and the S : N ratio of the excess output of urinary N and S is that of body tissue, showing a marked tissue catabolism, the faecal N output is lower than in the succeeding period on low-temperature dried blood meal. The following figures abstracted from Table XXV furnish conclusive evidence of the serious effect of high temperature on the nutritive value of blood meal proteins.

| Ration | Cow 1 | Cow 2 | Mean (uncorrected) | Mean (corrected) |
|---|---|---|---|---|
| Blood meal (high temp.) | 60·6, 65·3 | 57·5 | 61·1 | 57·5 |
| Blood meal (low temp.) | 75·1, 80·1, 80·6 | 76·8, 80·6, 84·5 | 79·6 | 75·0 |

### III. The supplementary relations of food proteins

It has long been known that the nutritive value of a mixture of proteins is not necessarily the mean of the biological values of each separately, i.e. certain proteins are capable of supplementing one another. This will naturally depend on the amino-acid deficiencies occurring; if proteins have different deficiencies they will always have some supplementary value. It may also be noted that a protein of high value is of little use as regards supplementing deficient proteins, whereas one of low biological value will have a potentially greater supplementary value. Thus decorticated earth-nut cake with a biological value of 49 has an available supplementing value of 51, whereas fresh spring grass with a biological value of 83 has only an available supplementing value of 17. To prove completely a supplementing value it is essential to feed each protein separately at the same level and finally both together, also at the same level of intake.

Much work on the supplementary value of proteins has been done on small animals (20, 21, 22, 23), but so far no experiment has been done with ruminants. In the present investigation, evidence is available to show that with milking cows two proteins with different amino-acid deficiencies can supplement one another. With cows 3–5 short periods were included in which decorticated earth-nut cake, known to be deficient in lysine (2, 12) and ossein, a protein extracted from bone which is deficient in tryptophane, were fed separately and together. Fig. 1 shows the effect of these rations on the milk yield. It is clear that, whereas each separately causes a marked fall in milk yield, both together give an increased production of milk. Similar results are obtained for the total N output of urine and milk (Figs. 4–6). The milk N falls markedly when each ration is fed separately, while the urinary N output increases; when both are fed together (at the same level) the milk N output increases and the urinary output falls markedly. With the N metabolism (Tables VII, VIII, XIV, XVIII, XXII) the same effect is seen, the high percentage of ammonia and low urea percentage becoming normal when both foods are mixed and fed together; the creatine output diminishes markedly and S : N ratio of the excess output disappears entirely. Unfortunately the periods during which the foods, separately or together, were fed were not of long enough duration to calculate the biological values.

### Summary

1. As regards their nutritive value for milk production, the proteins of spring grass are markedly superior to those of autumn grass. This is shown not only from a study of milk yield, but from the increased excretion of N and S in the urine, the increased creatine excretion, and the lower biological value given by autumn grass.

2. A study of the N and S metabolism of cows receiving fresh and artificially dried grass fails to reveal any significant differences in the nutritive

value as a result of artificial drying. There are no significant differences between the biological values of the proteins of fresh and of artificially dried autumn grass. A comparison of the mean biological values of the proteins of fresh and of artificially dried spring grass indicates that the latter has an inferior value for milk production, but the differences largely disappear when the biological values are calculated to a common standard.

3. No significant differences were found between the nutritive values of the proteins of three types of grass silage. The three types of silage, which were made from young summer grass, showed biological values equivalent to that of fresh spring grass.

4. It is shown that the proteins of blood meal prepared by a high-temperature process have a lower nutritive value than those of blood meal prepared by a low-temperature process.

5. Assuming the proteins of low-temperature dried blood meal to have an arbitrary biological value of 75, the comparative values for the other foodstuffs studied in this and in the previous investigations(1, 2) may be roughly classed as follows:

| | |
|---|---|
| 75–80 per cent. | Fresh and dried spring grass. |
| | Grass silage (made from summer grass). |
| | Low-temperature dried blood meal. |
| 60–65 per cent. | Fresh and dried autumn grass. |
| | Bean meal and pea meal. |
| 55–60 per cent. | High-temperature dried blood meal. |
| | Meat meal. |
| 50–55 per cent. | Decorticated earth-nut cake. |
| | Decorticated earth-nut cake *plus* flaked maize. |
| 45–50 per cent. | Linseed cake. |
| | Linseed-oil meal. |

These values refer to the complete rations as fed, and not to the individual foodstuffs.

6. The supplementary value of two poor quality proteins, one deficient in lysine and the other in tryptophane, has been demonstrated.

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## APPENDIX

### Table I. *Details of daily rations*

Cows 1 and 2

| Period | |
|---|---|
| | 4·55 kg. straw<br>1·50 kg. beet pulp } Maintenance ration constant throughout experiment<br>1·70 kg. oats |
| I | 5·00 kg. oats<br>3·75 kg. blood meal (high temp. dried) |
| II (*a*) | 5·00 kg. oats<br>3·75 kg. blood meal (low temp. dried) |
| (*b*) | 4·00 kg. oats<br>3·07 kg. blood meal (low temp. dried) |
| III | 4·10 kg. oats<br>3·07 kg. flaked maize<br>10·23 kg. grass |
| IV | 4·00 kg. oats<br>3·07 kg. blood meal (low temp. dried) |
| V | 4·00 kg. oats<br>3·07 kg. blood meal (high temp. dried) |

*Note.* Cow 2 stopped in period IV owing to injury to udder.

### Table II. *Details of daily rations*

Maintenance ration fed throughout to all animals:
4·55 kg. straw
1·50 kg. beet pulp
1·70 kg. oats

| Period | Production rations | Cows 3 and 4 | Cow 5 |
|---|---|---|---|
| 1 | Blood meal | 4·545 kg. oats<br>3·410 kg. blood-meal mixture | 3·300 kg. oats<br>2·700 kg. blood-meal mixture |
| 2 | Ossein | 4·545 kg. oats<br>3·410 kg. ossein mixture | 3·300 kg. oats<br>2·700 kg. ossein mixture |
| 3 | Ossein + decorticated earth-nut cake | 4·545 kg. oats<br>3·410 kg. ossein mixture<br>0·300 kg. decorticated earth-nut cake | 3·300 kg. oats<br>2·700 kg. ossein mixture<br>0·250 kg. decorticated earth-nut cake |
| 4 | Autumn grass | 5·100 kg. oats<br>3·070 kg. maize<br>10·230 kg. grass | 2·700 kg. oats<br>2·160 kg. maize<br>8·000 kg. grass |
| 5 | Blood meal | As in period 1 | |
| 6 | Silage | 5·100 kg. oats | 2·700 kg. oats |
| 7 | | 3·070 kg. maize | 2·070 kg. maize |
| 8 | | 15·000 kg. silage (A.I.V.)<br>16·000 kg. silage (molasses)<br>20·000 kg. silage (ordinary) | 12·000 kg. silage (A.I.V.)<br>13·500 kg. silage (molasses)<br>18·000 kg. silage (ordinary) |
| 9 | Decorticated earth-nut cake | 5·100 kg. oats<br>0·500 kg. decorticated earth-nut cake<br>1·000 kg. starch | 3·300 kg. oats<br>0·400 kg. decorticated earth-nut cake<br>0·750 kg. starch |
| 10 | Blood meal | As in period 1 | |

Table II A. *Details of daily rations*

| Period | Ration | Length days |
|---|---|---|
| 1 | Blood meal | 14 |
| 2 | Ossein | 8 |
| 3 | Ossein + decorticated earth-nut cake | 8 |
| 4 | Grass | 14 |
| 5 | Blood meal | 6 |
| 6 | A.I.V. silage* | 16 |
| 7 | Molasses silage | 14 |
| 8 | Ordinary silage† | 12 |
| | | 14 |
| 9 | Decorticated earth-nut cake | 8 |
| 10 | Blood meal‡ | 6 |
| | | 8 |

Period 2 (ossein) deficient in tryptophane.

Period 9 (decorticated earth-nut cake) deficient in lysine.

* Cow 4 would not eat A.I.V. silage, due to acidity.

† Cow 3 stopped ordinary silage after 12 days, due to the sudden appearance of a form of mastitis. Cows 4 and 5 continued for the complete 14 days.

‡ Cow 4 had only 6 days on blood meal. Cow 5 had 8 days.

Table III. *Details of daily rations*

Cow 6

| | | | |
|---|---|---|---|
| Maintenance: | | 4·550 kg. straw<br>1·500 kg. beet pulp<br>1·700 kg. oats | |
| Production: | Period 1. | 3·43 kg. oats<br>3·43 kg. beans<br>2·30 kg. maize | |
| | Period 2. | 3·43 kg. oats<br>22·50 kg. grass (fresh)<br>2·30 kg. maize | No straw eaten in this period |
| | Period 3. | 3·43 kg. oats<br>5·70 kg. dried grass<br>2·30 kg. maize | |
| | Period 4. | 3·43 kg. oats<br>3·43 kg. beans<br>2·30 kg. maize | |

Table IV. *Details of daily rations*

| Period | | |
|---|---|---|
| | 4·55 kg. straw<br>1·50 kg. beet pulp<br>1·70 kg. oats | Maintenance ration constant for all three animals |
| | Cows 7 and 8 | Cow 9 |
| 1 | 2·73 kg. oats<br>1·82 kg. maize<br>4·00 kg. spring-dried grass | 2·73 kg. oats<br>1·82 kg. maize<br>4·00 kg. spring-dried grass |
| 2 | 2·73 kg. oats<br>1·82 kg. maize<br>22·00 kg. fresh autumn grass | 2·73 kg. oats<br>1·82 kg. maize<br>2·75 kg. autumn-dried grass |
| 3 | Same as period 1 | Same as period 1 |
| 4 | 2·73 kg. oats<br>1·82 kg. maize<br>2·75 kg. autumn-dried grass | —<br>—<br>— |
| 5 | Same as period 1 | — |

Table V. *Urinary excretion of nitrogen* (*g. per 2-day period*)

| Period | Total N | Urea N | Ammonia N | Amino-acid N | Creatinine* | Creatine* |
|---|---|---|---|---|---|---|
| | | | Cow 1 | | | |
| Blood meal (high temp.) | 156·80 | 78·75 | 38·57 | 2·30 | 12·72 | 7·06 |
| Blood meal (low temp.) (*a*) | 103·63 | 59·12 | 28·42 | 2·43 | 11·50 | 2·87 |
| (*b*) | 112·62 | 63·44 | 27·09 | 3·49 | 10·37 | 3·89 |
| Spring grass | 108·01 | 60·32 | 28·35 | 5·36 | 9·62 | 2·95 |
| Blood meal (low temp.) | 104·67 | 59·97 | 22·78 | 2·29 | 9·85 | 2·63 |
| Blood meal (high temp.) | 167·03 | 72·04 | 50·11 | 2·45 | 10·97 | 8·00 |
| | | | Cow 2 | | | |
| Blood meal (high temp.) | 157·21 | 90·78 | 35·90 | 2·18 | 11·94 | 5·93 |
| Blood meal (low temp.) (*a*) | 96·96 | 51·95 | 24·50 | 1·67 | 10·09 | 4·23 |
| (*b*) | 104·75 | 60·00 | 29·68 | 3·63 | 8·48 | 2·54 |
| Spring grass | 110·06 | 69·13 | 32·74 | 4·38 | 10·34 | 2·65 |
| Blood meal (low temp.) | 118·24 | 73·14 | 19·14 | 2·58 | 10·19 | 2·78 |

Table VI. *Urinary partition of nitrogen* (*as percentage of total urinary nitrogen*)

| Period | Urea N | Ammonia N | Amino-acid N |
|---|---|---|---|
| | Cow 1 | | |
| Blood meal (high temp.) | 50·2 | 24·6 | 1·5 |
| Blood meal (low temp.) (*a*) | 56·7 | 27·3 | 2·4 |
| (*b*) | 56·1 | 24·0 | 3·1 |
| Spring grass | 55·8 | 26·3 | 5·0 |
| Blood meal (low temp.) | 57·1 | 21·7 | 2·2 |
| Blood meal (high temp.) | 43·1 | 29·9 | 1·5 |
| | Cow 2 | | |
| Blood meal (high temp.) | 57·9 | 22·9 | 1·4 |
| Blood meal (low temp.) (*a*) | 53·6 | 25·3 | 1·7 |
| (*b*) | 57·2 | 28·6 | 3·5 |
| Spring grass | 62·8 | 29·8 | 4·0 |
| Blood meal (low temp.) | 61·9 | 16·2 | 2·2 |

Table VII. *Urinary excretion of nitrogen* (*g. per 2-day period*)

| Period | Total N | Urea N | Ammonia N | Amino-acid N | Preformed creatinine* | Creatine* |
|---|---|---|---|---|---|---|
| | | | Cow 3 | | | |
| Blood meal | 97·75 | 50·92 | 22·28 | 5·50 | 9·04 | 4·37 |
| Ossein | 166·50 | 71·28 | 54·17 | 3·40 | 9·09 | 6·65 |
| Ossein + decorticated earth-nut cake | 97·67 | 54·72 | 26·91 | 2·03 | 8·30 | 3·90 |
| Autumn grass | 122·25 | 51·80 | 49·83 | 4·81 | 7·15 | 7·50 |
| Blood meal | 95·53 | — | — | — | — | — |
| A.I.V. silage | 108·53 | 49·03 | 39·77 | 1·50 | 11·54 | 6·10 |
| Molasses silage | 78·47 | 47·33 | 18·89 | 1·57 | 10·48 | 4·87 |
| Ordinary silage | 74·06 | 33·43 | 22·76 | 2·58 | 9·08 | 5·25 |
| | | | Cow 4 | | | |
| Blood meal | 115·10 | 67·91 | 22·13 | 4·87 | 9·84 | 2·93 |
| Ossein | 139·20 | 58·00 | 50·07 | 3·01 | 9·93 | 4·63 |
| Ossein + decorticated earth-nut cake | 112·18 | 58·82 | 23·02 | 3·63 | 9·47 | 1·91 |
| Autumn grass | 142·06 | 62·01 | 64·15 | 4·76 | 6·98 | 4·06 |
| Blood meal | 88·88 | — | — | — | — | — |
| A.I.V. silage | — | — | — | — | — | — |
| Molasses silage | 70·04 | 36·13 | 15·90 | 1·75 | 8·73 | 4·17 |
| Ordinary silage | 76·02 | 36·38 | 21·53 | 2·21 | 8·51 | 4·64 |

* Not given as N.

Table VII (*continued*)

| Period | Total N | Urea N | Ammonia N | Amino-acid N | Preformed creatinine* | Creatine* |
|---|---|---|---|---|---|---|
| | | | Cow 5 | | | |
| Blood meal | 119·65 | 64·25 | 24·76 | 3·95 | 8·79 | 2·64 |
| Ossein | 153·57 | 94·40 | 40·96 | 5·55 | 12·88 | 5·40 |
| Ossein + decorticated earth-nut cake | 119·05 | 70·93 | 24·34 | 3·96 | 12·59 | 5·95 |
| Autumn grass | 144·04 | 55·88 | 66·00 | 4·49 | 8·93 | 7·22 |
| Blood meal | 127·29 | — | — | — | — | — |
| A.I.V. silage | 110·64 | 51·70 | 32·22 | 2·50 | 10·58 | 5·98 |
| Molasses silage | 80·10 | 53·21 | 11·22 | 1·48 | 9·38 | 3·78 |
| Ordinary silage | 82·57 | 43·55 | 14·65 | 2·66 | 11·08 | 3·62 |

Table VIII. *Urinary partition of nitrogen (as percentage of total nitrogen)*

| Period | Urea N | Ammonia N | Amino-acid N |
|---|---|---|---|
| Cow 3 | | | |
| Blood meal | 52·1 | 22·8 | 5·6 |
| Ossein | 42·7 | 32·4 | 2·0 |
| Ossein + decorticated earth-nut cake | 56·0 | 27·5 | 2·0 |
| Autumn grass | 42·4 | 40·8 | 4·0 |
| Blood meal | — | — | — |
| A.I.V. silage | 45·0 | 36·7 | 1·4 |
| Molasses silage | 60·2 | 24·2 | 2·0 |
| Ordinary silage | 45·1 | 30·8 | 3·5 |
| Cow 4 | | | |
| Blood meal | 59·2 | 19·2 | 4·2 |
| Ossein | 41·7 | 36·0 | 2·2 |
| Ossein + decorticated earth-nut cake | 52·7 | 20·5 | 3·2 |
| Autumn grass | 43·7 | 45·1 | 3·4 |
| Blood meal | — | — | — |
| A.I.V. silage | — | — | — |
| Molasses silage | 51·6 | 22·7 | 2·5 |
| Ordinary silage | 48·0 | 28·3 | 2·9 |
| Cow 5 | | | |
| Blood meal | 53·5 | 20·6 | 3·3 |
| Ossein | 61·3 | 26·6 | 3·6 |
| Ossein + decorticated earth-nut cake | 59·6 | 20·4 | 3·3 |
| Autumn grass | 38·9 | 45·8 | 3·1 |
| Blood meal | — | — | — |
| A.I.V. silage | 47·0 | 29·3 | 2·3 |
| Molasses silage | 66·5 | 14·0 | 1·9 |
| Ordinary silage | 52·8 | 17·8 | 3·2 |

Table IX. *Urinary excretion of nitrogen (g. per 2-day period)*

| Period | Total N | Urea N | Ammonia N | Amino-acid N | Creatinine* | Creatine* |
|---|---|---|---|---|---|---|
| | | | Cow 6 | | | |
| Beans | 103·92 | 68·33 | 17·11 | 3·41 | 9·34 | 3·18 |
| Fresh spring grass | 107·27 | 60·67 | 23·24 | 2·95 | 9·26 | 3·50 |
| Dried spring grass | 100·59 | 71·86 | 20·26 | 5·24 | 9·08 | 5·50 |
| Beans | 147·16 | 83·82 | 36·62 | 3·56 | 8·77 | 5·52 |

* Not given as N.

Table X. *Urinary partition of nitrogen (as percentage of total urinary nitrogen)*

| Period | Urea N | Ammonia N | Amino-acid N |
|---|---|---|---|
| | Cow 6 | | |
| Beans | 65·8 | 16·5 | 3·3 |
| Fresh spring grass | 60·5 | 21·7 | 2·8 |
| Dried spring grass | 71·5 | 20·2 | 5·2 |
| Beans | 57·1 | 24·9 | 2·4 |

Table XI. *Urinary excretion of nitrogen (g. per 2-day period)*

| Period | Total N | Urea N | Ammonia N | Amino-acid N | Creatinine* | Creatine* |
|---|---|---|---|---|---|---|
| | | Cow 7 | | | | |
| Spring-dried grass | 94·41 | 47·84 | 22·11 | 2·74 | 9·18 | 2·87 |
| Fresh autumn grass | 150·78 | 70·02 | 37·21 | 1·86 | 7·22 | 6·39 |
| Spring-dried grass | 102·64 | 46·15 | 25·70 | 2·70 | 7·44 | 2·99 |
| Autumn-dried grass | 119·46 | 47·97 | 31·00 | 3·22 | 8·40 | 3·49 |
| Spring-dried grass | 102·39 | 45·73 | 26·74 | 6·35 | 7·80 | 3·57 |
| | | Cow 8 | | | | |
| Spring-dried grass | 116·53 | 59·84 | 23·89 | 3·88 | 9·54 | 3·98 |
| Fresh autumn grass | 132·25 | 54·03 | 48·92 | 2·41 | 7·67 | 5·23 |
| Spring-dried grass | 109·58 | 46·55 | 18·85 | 5·60 | 6·28 | 3·24 |
| Autumn-dried grass | 123·76 | 50·19 | 36·06 | 3·47 | 7·78 | 4·75 |
| Spring-dried grass | 110·59 | 54·13 | 29·72 | 6·18 | 7·76 | 3·01 |
| | | Cow 9 | | | | |
| Spring-dried grass | 109·19 | 52·01 | 25·59 | 6·37 | 8·95 | 4·89 |
| Autumn-dried grass | 129·16 | 53·73 | 37·74 | 6·88 | 7·47 | 4·58 |
| Spring-dried grass | 124·84 | 62·14 | 27·04 | 6·22 | 8·04 | 4·11 |

Table XII. *Urinary partition of nitrogen (expressed as percentage of total urinary nitrogen)*

| Period | Urea N | Ammonia N | Amino-acid N |
|---|---|---|---|
| | Cow 7 | | |
| Spring-dried grass | 50·6 | 23·4 | 2·9 |
| Fresh autumn grass | 46·4 | 24·7 | 1·2 |
| Spring-dried grass | 45·0 | 25·0 | 2·6 |
| Autumn-dried grass | 40·1 | 25·9 | 2·7 |
| Spring-dried grass | 44·7 | 26·1 | 6·2 |
| | Cow 8 | | |
| Spring-dried grass | 51·3 | 20·5 | 3·3 |
| Fresh autumn grass | 40·9 | 37·0 | 1·8 |
| Spring-dried grass | 42·5 | 17·2 | 5·1 |
| Autumn-dried grass | 40·5 | 29·1 | 2·8 |
| Spring-dried grass | 48·9 | 26·9 | 5·6 |
| | Cow 9 | | |
| Spring-dried grass | 47·6 | 23·4 | 5·8 |
| Autumn-dried grass | 41·6 | 29·2 | 5·3 |
| Spring-dried grass | 49·8 | 21·6 | 5·0 |

* Not given as N.

Table XIII. *Nitrogen and sulphur output in urine (g. per 2-day period)*

| Period | Urinary N | Urinary S | Excess N | Excess S | S : N ratio of excess |
|---|---|---|---|---|---|
| | | Cow 1 | | | |
| Blood meal (high temp.) | 156·80 | 18·74 | 53·17 | 3·50 | 1 : 15·05 |
| Blood meal (low temp.) (*a*) | 103·63 | 15·24 | — | — | — |
| (*b*) | 112·62 | 15·85 | — | — | — |
| Spring grass | 108·01 | 23·65 | — | — | — |
| Blood meal (low temp.) | 104·67 | 16·02 | — | — | — |
| Blood meal (high temp.) | 167·03 | 19·02 | 63·40 | 3·78 | 1 : 16·8 |
| | | Cow 2 | | | |
| Blood meal (high temp.) | 157·21 | 16·28 | 60·25 | 3·71 | 1 : 16·2 |
| Blood meal (low temp.) (*a*) | 96·96 | 12·57 | — | — | — |
| (*b*) | 104·75 | 12·87 | — | — | — |
| Spring grass | 110·06 | 21·77 | — | — | — |
| Blood meal (low temp.) | 118·24 | 13·33 | — | — | — |

Table XIV. *Nitrogen and sulphur output in urine (g. per 2-day period)*

| Period | Urinary N | Urinary S | Excess N | Excess S | S : N ratio of excess |
|---|---|---|---|---|---|
| | | Cow 3 | | | |
| Blood meal | 97·75 | 22·35 | — | — | — |
| Ossein | 166·50 | 34·23 | 68·75 | 11·88 | 1 : 5·9 |
| Ossein + decorticated earth-nut cake | 97·67 | 20·92 | — | — | — |
| Autumn grass | 122·25 | 39·32 | 24·50 | 1·60 | 1 : 15·3 |
| Blood meal | 95·53 | — | — | — | — |
| A.I.V. silage | 108·53 | 34·60 | 10·78 | 12·25 | 1 : 0·9 |
| Molasses silage | 78·47 | 20·97 | — | — | — |
| Ordinary silage | 74·06 | 19·38 | — | — | — |
| | | Cow 4 | | | |
| Blood meal | 115·10 | 22·84 | — | — | — |
| Ossein | 139·20 | 26·68 | 24·10 | 3·84 | 1 : 6·5 |
| Ossein + decorticated earth-nut cake | 112·18 | 19·80 | — | — | — |
| Autumn grass | 142·06 | 34·81 | 26·96 | 1·13 | 1 : 23·8 |
| Blood meal | 88·88 | — | — | — | — |
| A.I.V. silage | — | — | — | — | — |
| Molasses silage | 70·04 | 14·45 | — | — | — |
| Ordinary silage | 76·02 | 15·67 | — | — | — |
| | | Cow 5 | | | |
| Blood meal | 119·65 | 22·91 | — | — | — |
| Ossein | 153·57 | 30·26 | 33·92 | 7·35 | 1 : 4·7 |
| Ossein + decorticated earth-nut cake | 119·05 | 20·07 | — | — | — |
| Autumn grass | 144·04 | 42·05 | 24·39 | 1·75 | 1 : 14·0 |
| Blood meal | 127·29 | — | — | — | — |
| A.I.V. silage | 110·64 | 33·31 | — | — | — |
| Molasses silage | 80·10 | 17·81 | — | — | — |
| Ordinary silage | 82·57 | 17·86 | — | — | — |

Table XV. *Nitrogen and sulphur output in urine (g. per 2-day period)*

| Ration | Urinary N | Urinary S | S : N ratio of excess |
|---|---|---|---|
| | Cow 6 | | |
| Beans | 103·92 | 10·75 | 1 : 9·7 |
| Fresh spring grass | 107·27 | 18·63 | 1 : 5·8 |
| Dried spring grass | 100·57 | 21·90 | 1 : 4·6 |
| Beans | 147·16 | 16·35 | 1 : 9·0 |

Table XVI. *Nitrogen and sulphur output in urine (g. per 2-day output)*

| Period | Urinary N | Urinary S | Excess N | Excess S | S : N ratio of excess |
|---|---|---|---|---|---|
| | | Cow 7 | | | |
| Spring-dried grass | 94·41 | 22·69 | 0·0 | 0·0 | — |
| Fresh autumn grass | 150·78 | 26·04 | 56·37 | 3·35 | 1 : 16·82 |
| Autumn-dried grass | 119·46 | 30·33 | 25·05 | 7·64 | 1 : 3·28 |
| Spring-dried grass | 102·39 | 23·25 | 0·0 | 0·0 | — |
| | | Cow 8 | | | |
| Spring-dried grass | 116·53 | 23·20 | 0·0 | 0·0 | — |
| Fresh autumn grass | 132·25 | 24·11 | 15·72 | 0·91 | 1 : 17·27 |
| Autumn-dried grass | 123·76 | 26·31 | 7·23 | 3·11 | 1 : 2·33 |
| Spring-dried grass | 110·59 | 23·14 | 0·0 | 0·0 | — |
| | | Cow 9 | | | |
| Spring-dried grass | 109·19 | 22·39 | 0·0 | 0·0 | — |
| Autumn-dried grass | 129·16 | 29·85 | 19·97 | 7·46 | 1 : 2·68 |
| Spring-dried grass | 124·84 | 24·16 | 0·0 | 0·0 | — |

Table XVII. *Nitrogen balances for each complete feeding period*

| Period | Intake g. | Total output g. | Gain or loss g. | Daily gain or loss g. |
|---|---|---|---|---|
| | | Cow 1 | | |
| Blood meal (high temp.) | 3580·00 | 3632·68 | − 52·68 | − 3·76 |
| Blood meal (low temp.) (*a*) | 4091·48 | 4026·31 | + 65·17 | + 8·15 |
| (*b*) | 2445·45 | 2417·71 | + 27·74 | + 2·77 |
| Spring grass | 6547·40 | 6478·32 | + 69·08 | + 2·47 |
| Blood meal (low temp.) | 4677·00 | 4547·27 | +129·73 | + 6·49 |
| Blood meal (high temp.) | 2338·50 | 2383·62 | − 45·12 | − 4·51 |
| | | Cow 2 | | |
| Blood meal (high temp.) | 3580·00 | 3502·76 | + 77·24 | + 5·52 |
| Blood meal (low temp.) (*a*) | 4091·48 | 4090·33 | + 1·15 | + 0·07 |
| (*b*) | 2445·45 | 2334·74 | +110·71 | +11·07 |
| Spring grass | 6547·40 | 6394·80 | +152·60 | + 5·45 |
| Blood meal (low temp.) | 2806·20 | 2781·50 | + 24·70 | + 2·06 |

Table XVIII. *Nitrogen balances for each complete feeding period*

| Period | Intake g. | Output g. | Gain or loss g. | Daily gain or loss g. |
|---|---|---|---|---|
| | Cow 3 | | | |
| Blood meal | 3150·00 | 3106·46 | + 43·54 | + 3·11 |
| Ossein | 1800·00 | 1983·56 | −183·56 | −22·95 |
| Ossein + decorticated earth-nut cake | 1800·00 | 1751·60 | + 48·40 | + 6·05 |
| Autumn grass | 3150·00 | 3044·58 | +105·42 | + 7·53 |
| Blood meal | 1350·00 | 1341·18 | + 8·82 | + 1·47 |
| A.I.V. silage | 3600·00 | 3628·64 | − 28·64 | − 1·79 |
| Molasses silage | 3150·00 | 2899·19 | +250·81 | +17·92 |
| Ordinary silage | 2341·56 | 2316·90 | + 24·66 | + 2·06 |
| | Cow 4 | | | |
| Blood meal | 3150·00 | 3566·92 | −416·92 | −29·78 |
| Ossein | 1800·00 | 1794·64 | + 5·36 | + 0·67 |
| Ossein + decorticated earth-nut cake | 1800·00 | 1791·44 | + 8·56 | + 1·07 |
| Autumn grass | 3150·00 | 3005·52 | +144·48 | +10·32 |
| Blood meal | 1350·00 | 1197·12 | +152·88 | +25·48 |
| A.I.V. silage | — | — | — | — |
| Molasses silage | 2625·00 | 2555·28 | + 69·72 | + 4·98 |
| Ordinary silage | 2250·00 | 2123·82 | +126·18 | +10·52 |
| Decorticated earth-nut cake | 1500·00 | 1545·16 | − 45·16 | − 5·65 |
| Blood meal | 1125·00 | 1049·19 | + 75·81 | +12·64 |

Table XVIII (*continued*)

| Period | Intake g. | Output g. | Gain or loss g. | Daily gain or loss g. |
|---|---|---|---|---|
| | Cow 5 | | | |
| Blood meal | 2819·04 | 2953·44 | −134·40 | − 9·66 |
| Ossein | 1610·88 | 1673·12 | − 62·24 | − 7·78 |
| Ossein + decorticated earth-nut cake | 1610·88 | 1704·64 | − 93·76 | −11·72 |
| Autumn grass | 2819·04 | 2744·84 | + 74·20 | + 5·30 |
| Blood meal | 1208·16 | 1201·53 | + 6·63 | + 1·11 |
| A.I.V. silage | 3221·76 | 2924·24 | +297·52 | +18·60 |
| Molasses silage | 2819·04 | 2300·62 | +518·42 | +37·13 |
| Ordinary silage | 1982·16 | 1886·64 | + 95·52 | + 7·96 |
| Decorticated earth-nut cake | 1321·44 | 1357·16 | − 35·72 | − 4·45 |
| Blood meal | 1321·44 | 1349·32 | − 27·88 | − 3·49 |

Table XIX. *Nitrogen balances for each complete feeding period*

| Period | Intake g. | Output g. | Gain or loss g. | Daily gain or loss g. |
|---|---|---|---|---|
| | | Cow 6 | | |
| Beans | 5097 | 5117·20 | − 20·20 | +2·17 |
| Fresh grass | 5170 | 5077·25 | + 92·75 | +4·21 |
| Dried grass | 5090 | 4976·66 | +113·38 | +5·13 |
| Beans | 2780 | 2775·92 | + 4·08 | +0·34 |

Table XX. *Nitrogen balances for each complete period*

| Period | Intake g. | Total output g. | Gain or loss g. | Daily gain or loss g. |
|---|---|---|---|---|
| | | Cow 7 | | |
| Dried spring grass | 6544·56 | 6576·36 | −31·80 | −0·89 |
| Fresh autumn grass | 3172·28 | 3132·29 | +39·99 | +2·86 |
| Dried spring grass | 2180·20 | 2175·32 | + 4·88 | +0·49 |
| Dried autumn grass | 3964·36 | 3562·08 | +62·93 | +2·94 |
| Dried spring grass | 3568·32 | — | + 6·24 | +0·39 |
| | | Cow 8 | | |
| Dried spring grass | 8768·72 | 8794·35 | −25·63 | −0·71 |
| Fresh autumn grass | 3142·28 | 3075·54 | +66·74 | +4·77 |
| Dried spring grass | 2330·20 | 2270·25 | +59·95 | +6·00 |
| Dried autumn grass | 3554·36 | 3542·82 | +11·54 | +0·64 |
| Dried spring grass | 3548·32 | 3561·28 | −12·96 | −0·81 |
| | | Cow 9 | | |
| Dried spring grass | 4455·00 | 4470·18 | −15·18 | −0·69 |
| Dried autumn grass | 2635·00 | 2614·21 | +20·79 | +1·49 |
| Dried spring grass | 2865·00 | 2882·68 | −17·68 | −1·26 |

Table XXI. *Faecal nitrogen output* (*g. per 2-day period*)

| Period | Cow 1 | Cow 2 |
|---|---|---|
| Blood meal (high temp.) | 167·33 | 146·30 |
| Blood meal (low temp.) (*a*) | 217·84 | 234·46 |
| (*b*) | 217·57 | 210·98 |
| Spring grass | 200·83 | 199·09 |
| Blood meal (low temp.) | 196·75 | 199·10 |
| Blood meal (high temp.) | 167·78 | — |

Table XXII. *Faecal nitrogen output* (*g. per 2-day period*)

| Period | Cow 3 | Cow 4 | Cow 5 |
|---|---|---|---|
| Blood meal | 186·80 | 192·92 | 181·40 |
| Ossein | 186·09 | 174·68 | 153·56 |
| Ossein + decorticated earth-nut cake | 193·50 | 192·75 | 200·66 |
| Autumn grass | 179·80 | 165·14 | 158·94 |
| Blood meal | 201·53 | 178·36 | 177·22 |
| A.I.V. silage | 194·92 | — | 191·94 |
| Molasses silage | 218·59 | 184·26 | 198·49 |
| Ordinary silage | 169·27 | 165·76 | 160·94 |
| Decorticated earth-nut cake | — | 156·72 | 124·75 |
| Blood meal | — | 166·99 | 173·53 |

Table XXIII. *Faecal nitrogen output* (*g. per 2-day period*)

| Period | Cow 6 |
|---|---|
| Beans | 192·13 |
| Fresh spring grass | 204·05 |
| Dried spring grass | 209·55 |
| Beans | 188·11 |

Table XXIV. *Faecal nitrogen output* (*g. per 2-day period*)

| Period | Cow 7 | Cow 8 | Cow 9 |
|---|---|---|---|
| Dried spring grass | 194·58 | 199·38 | — |
| Fresh autumn grass | 147·84 | 164·43 | — |
| Dried spring grass | 179·19 | 186·34 | 197·71 |
| Dried autumn grass | 158·60 | 161·64 | 165·27 |
| Dried spring grass | 188·73 | 198·10 | 192·05 |

Table XXV. *Biological values*

| Period | % decrease in milk N from first period | $X$ | $I_{(corr.)}$ | $F_t$ | $M_t$ | $F_m$ | $M_m$ | $F_x$ | $M_x$ | Biological value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cow 1 | | | | | | |
| Dried blood meal (high temp.) | 0·0 | 194·84 | 400 | 167·33 | 107·58 | 78·68 | 59·68 | 0·00 | 0·00 | 60·6 |
| Dried blood meal (low temp.) (*a*) | 7·0 | 181·82 | 372 | 217·84 | 107·58 | 78·68 | 59·68 | 3·64 | 2·41 | 75·1 |
| (*b*) | 17·0 | 153·35 | 332 | 217·57 | 99·00 | 78·68 | 59·68 | 11·12 | 8·00 | 80·1 |
| Fresh spring grass | 22·0 | 153·90 | 312 | 200·83 | 99·00 | 78·68 | 59·68 | 11·12 | 8·00 | 81·7 |
| Blood meal (low temp.) | 22·5 | 153·31 | 310 | 196·75 | 99·00 | 78·68 | 59·68 | 11·36 | 8·11 | 80·6 |
| Blood meal (high temp.) | 23·0 | 141·91 | 308 | 167·78 | 99·00 | 78·68 | 59·68 | 11·69 | 8·47 | 65·3 |
| | | | | Cow 2 | | | | | | |
| Blood meal (high temp.) | 0·0 | 196·89 | 400 | 146·30 | 107·58 | 78·68 | 59·68 | 0·00 | 0·00 | 57·6 |
| Blood meal (low temp.) (*a*) | 7·0 | 179·87 | 372 | 234·46 | 107·58 | 78·68 | 59·68 | 5·44 | 3·12 | 80·3 |
| (*b*) | 17·0 | 151·22 | 332 | 210·98 | 99·00 | 78·68 | 59·68 | 12·01 | 7·85 | 76·8 |
| Fresh spring grass | 25·0 | 147·62 | 300 | 199·09 | 99·00 | 78·68 | 59·68 | 13·17 | 8·46 | 83·4 |
| Blood meal (low temp.) | 26·0 | 146·25 | 296 | 199·10 | 99·00 | 78·68 | 59·68 | 13·69 | 8·97 | 84·5 |

Table XXVI. *Biological values*

| Period | % decrease in milk N from first period | $X$ | $I_{(corr.)}$ | $F_t$ | $M_t$ | $F_m$ | $M_m$ | $F_x$ | $M_x$ | Biological value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cow 3 | | | | | | |
| Blood meal | 0·0 | 159·23 | 312·0 | 186·80 | 103·62 | 78·68 | 59·68 | 0·00 | 0·00 | 75·9 |
| Fresh autumn grass | 1·6 | 142·89 | 307·0 | 179·80 | 112·20 | 78·68 | 59·68 | 1·04 | 0·65 | 64·9 |
| A.I.V. silage | 4·8 | 150·13 | 297·0 | 194·92 | 112·20 | 78·68 | 59·68 | 1·76 | 1·03 | 77·2 |
| Molasses silage | 5·8 | 137·11 | 293·7 | 218·59 | 112·20 | 78·68 | 59·68 | 2·58 | 1·36 | 82·0 |
| Ordinary silage | 7·1 | 142·82 | 289·7 | 169·27 | 82·20 | 78·68 | 59·68 | 2·98 | 1·58 | 78·1 |
| | | | | Cow 4 | | | | | | |
| Blood meal | 0·0 | 173·13 | 312·0 | 192·92 | 103·62 | 78·68 | 59·68 | 0·00 | 0·00 | 84·9 |
| Fresh autumn grass | 18·0 | 121·87 | 255·9 | 165·14 | 112·20 | 78·68 | 59·68 | 8·14 | 4·22 | 67·7 |
| A.I.V. silage | — | — | — | — | 112·20 | 78·68 | 59·68 | — | — | — |
| Molasses silage | 22·1 | 110·74 | 243·1 | 184·26 | 112·20 | 78·68 | 59·68 | 14·75 | 7·73 | 76·3 |
| Ordinary silage | 24·1 | 112·19 | 236·8 | 165·76 | 82·20 | 78·68 | 59·68 | 15·97 | 8·44 | 88·5 |
| | | | | Cow 5 | | | | | | |
| Blood meal | 0·0 | 120·87 | 265·0 | 181·40 | 90·80 | 78·68 | 59·68 | 0·00 | 0·00 | 77·8 |
| Fresh autumn grass | 10·3 | 89·14 | 237·6 | 158·94 | 90·80 | 78·68 | 59·68 | 8·01 | 4·14 | 60·8 |
| A.I.V. silage | 15·3 | 82·95 | 224·4 | 191·94 | 90·80 | 78·68 | 59·68 | 13·26 | 7·51 | 84·2 |
| Molasses silage | 19·4 | 70·07 | 213·5 | 198·49 | 90·80 | 78·68 | 59·68 | 17·20 | 8·59 | 79·4 |
| Ordinary silage | 21·4 | 70·93 | 208·1 | 160·94 | 70·80 | 78·68 | 59·68 | 21·39 | 12·72 | 78·5 |

Table XXVII. *Biological values*

| Period | % decrease in milk N from first period | $X$ | $I_{(corr.)}$ | $F_t$ | $M_t$ | $F_m$ | $M_m$ | $F_x$ | $M_x$ | Biological value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cow 6 | | | | | | |
| Bean meal | 0·0 | 169·15 | 350·0 | 192·13 | 111·60 | 78·68 | 59·68 | 0·00 | 0·00 | 67·5 |
| Fresh spring grass | 13·7 | 160·24 | 302·0 | 204·05 | 111·60 | 78·68 | 59·68 | 5·22 | 3·36 | 84·9 |
| Dried spring grass | 18·9 | 142·28 | 283·7 | 209·55 | 111·60 | 78·68 | 59·68 | 8·35 | 5·74 | 86·7 |
| Bean meal | 20·7 | 124·00 | 277·5 | 188·11 | 111·60 | 78·68 | 59·68 | 9·42 | 6·11 | 69·4 |

Table XXVIII. *Biological values*

| Period | % decrease in milk N from first period | $X$ | $I_{(corr.)}$ | $F_t$ | $M_t$ | $F_m$ | $M_m$ | $F_x$ | $M_x$ | Biological value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cow 7 | | | | | | |
| Dried spring grass | 0·0 | 133·61 | 252·00 | 194·58 | 107·58 | 78·68 | 59·68 | 0·00 | 0·00 | 72·6 |
| Fresh autumn grass | 5·4 | 118·85 | 238·04 | 147·84 | 107·58 | 78·68 | 59·68 | 2·79 | 1·74 | 52·2 |
| Dried spring grass | 7·7 | 123·23 | 232·05 | 179·19 | 107·58 | 78·68 | 59·68 | 3·99 | 2·50 | 68·1 |
| Dried autumn grass | 10·6 | 115·43 | 225·20 | 158·60 | 99·33 | 78·68 | 59·68 | 5·36 | 2·95 | 61·5 |
| Dried spring grass | 14·3 | 114·15 | 215·85 | 188·73 | 107·58 | 78·68 | 59·68 | 7·23 | 4·64 | 73·0 |
| | | | | Cow 8 | | | | | | |
| Dried spring grass | 0·0 | 142·67 | 262·00 | 199·38 | 107·58 | 78·68 | 59·68 | 0·00 | 0·00 | 75·4 |
| Fresh autumn grass | 10·8 | 122·68 | 233·70 | 164·43 | 107·58 | 78·68 | 59·68 | 5·66 | 3·54 | 62·0 |
| Dried spring grass | 13·5 | 128·14 | 226·55 | 186·34 | 107·58 | 78·68 | 59·68 | 7·09 | 4·46 | 75·6 |
| Dried autumn grass | 14·9 | 118·25 | 223·00 | 161·64 | 99·33 | 78·68 | 59·68 | 7·80 | 4·21 | 64·5 |
| Dried spring grass | 15·9 | 116·46 | 220·50 | 198·10 | 107·58 | 78·68 | 59·68 | 8·30 | 6·22 | 77·1 |
| | | | | Cow 9 | | | | | | |
| Dried spring grass | 0·0 | 139·48 | 252·00 | 197·71 | 107·58 | 86·42 | 51·15 | 0·00 | 0·00 | 70·8 |
| Dried autumn grass | 10·6 | 109·03 | 225·29 | 165·27 | 99·33 | 86·42 | 51·15 | 5·45 | 3·54 | 55·6 |
| Dried spring grass | 14·2 | 114·92 | 216·22 | 192·05 | 107·58 | 86·42 | 51·15 | 7·20 | 4·56 | 70·1 |

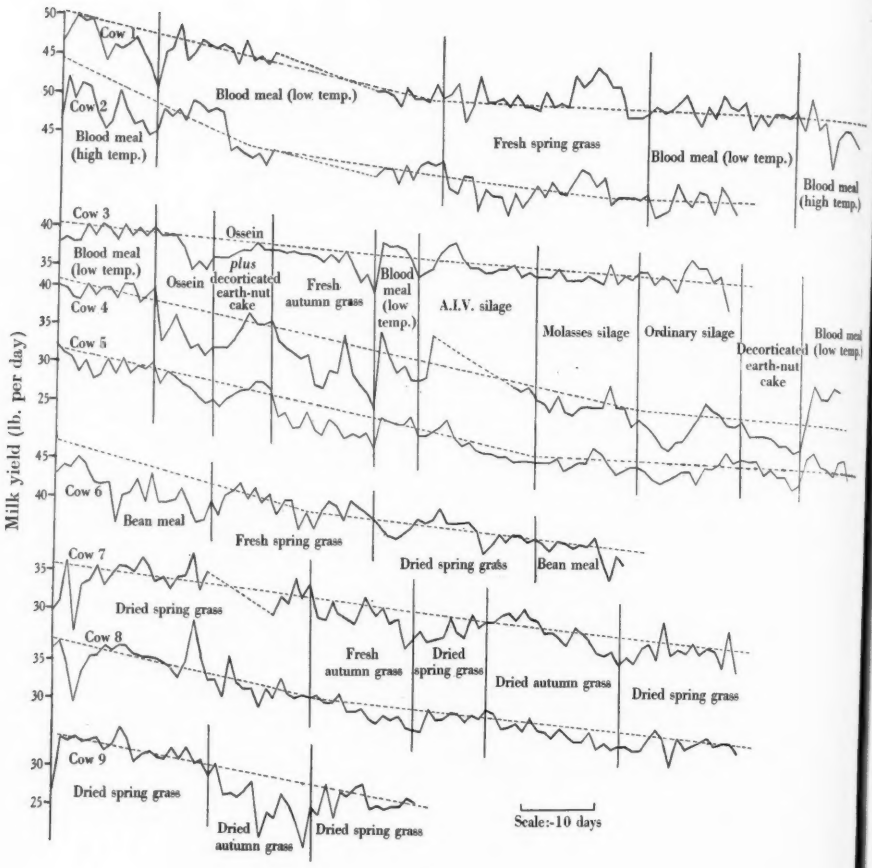
Milk yield (lb. per day)
Cow 1
Blood meal (low temp.)
Fresh spring grass
Blood meal (low temp.)
Blood meal (high temp.)
Cow 2
Blood meal (high temp.)
Cow 3
Ossein
Blood meal (low temp.)
Ossein plus decorticated earth-nut cake
Fresh autumn grass
Blood meal (low temp.)
A.I.V. silage
Molasses silage
Ordinary silage
Decorticated earth-nut cake
Cow 4
Cow 5
Cow 6
Bean meal
Fresh spring grass
Dried spring grass
Bean meal
Cow 7
Dried spring grass
Cow 8
Fresh autumn grass
Dried spring grass
Dried autumn grass
Dried spring grass
Cow 9
Dried spring grass
Dried autumn grass
Dried spring grass
Scale:-10 days

Fig. 1.

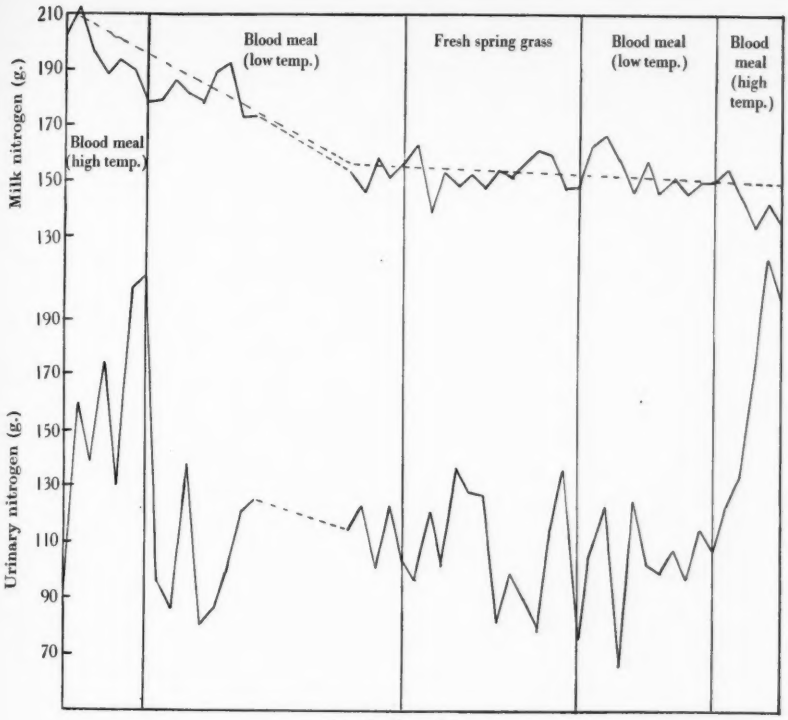


Fig. 2. (Cow 1.)

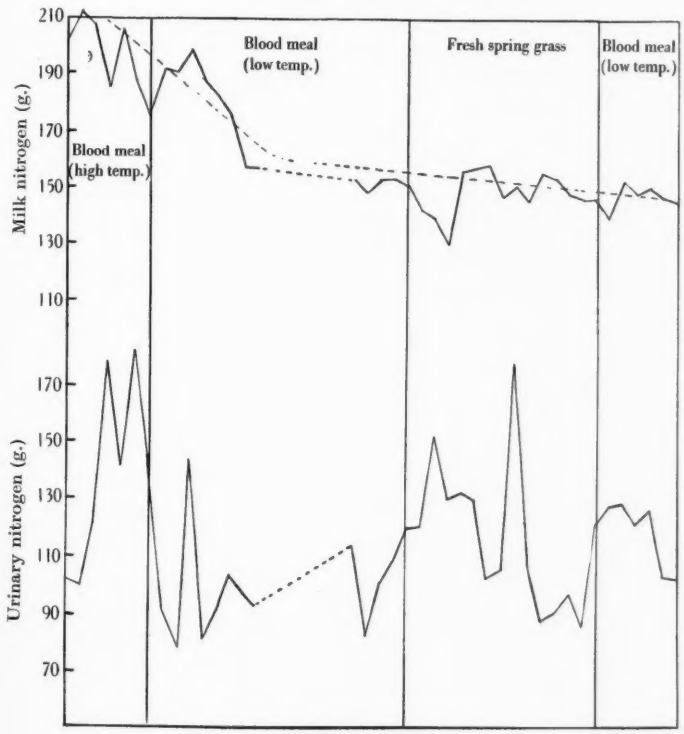


Fig. 3. (Cow 2.)

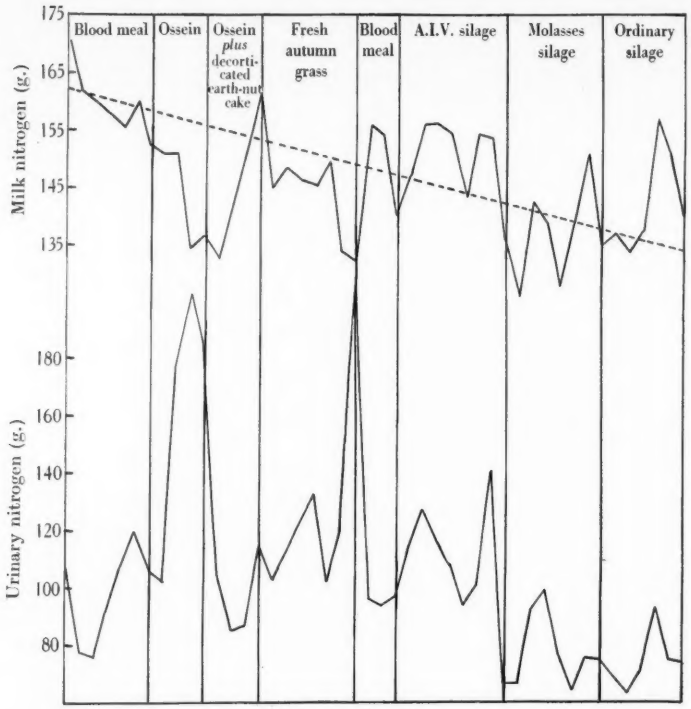


Fig. 4. (Cow 3.)

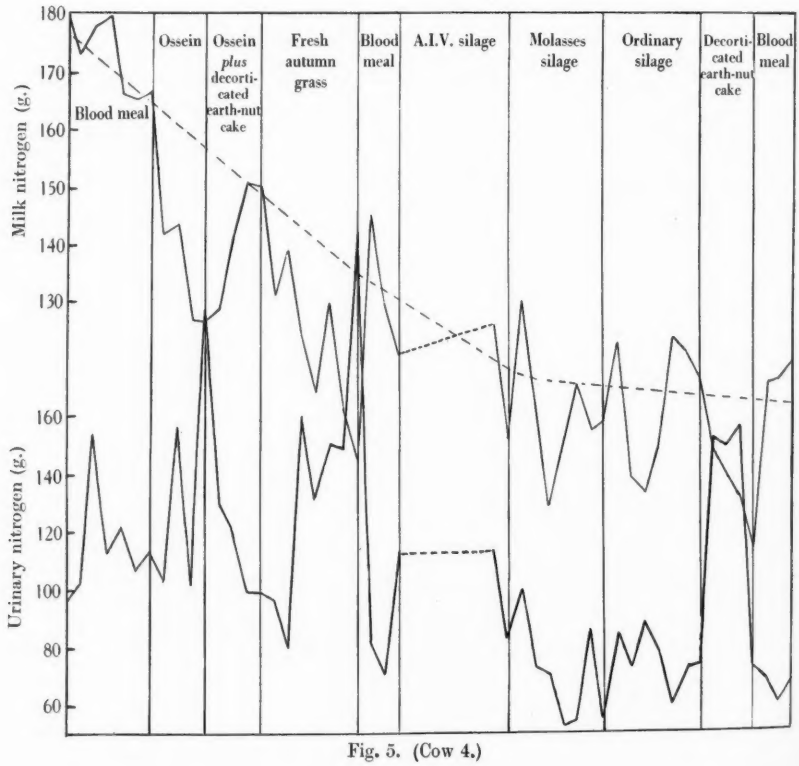


Fig. 5. (Cow 4.)

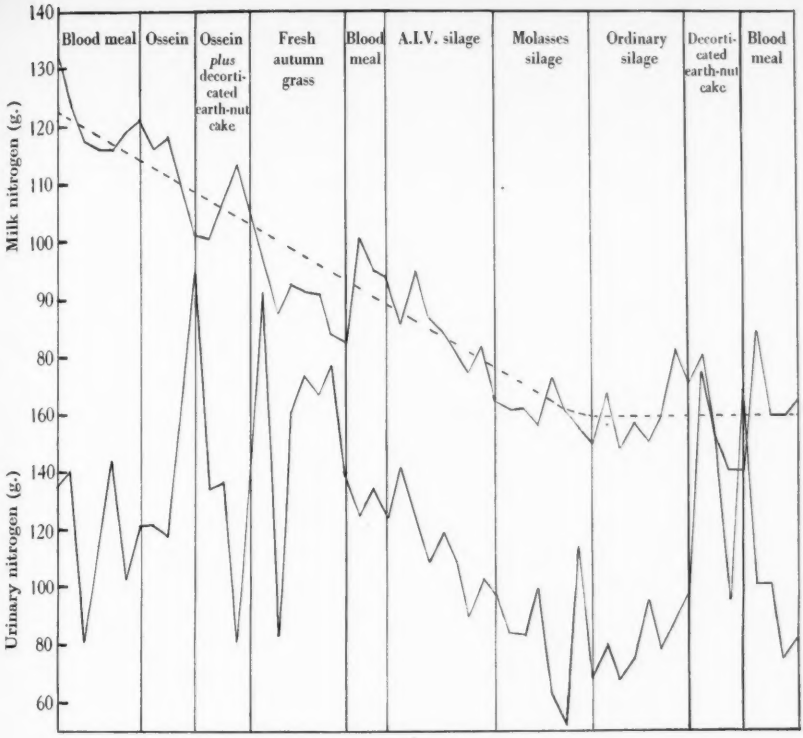


Fig. 6. (Cow 5.)

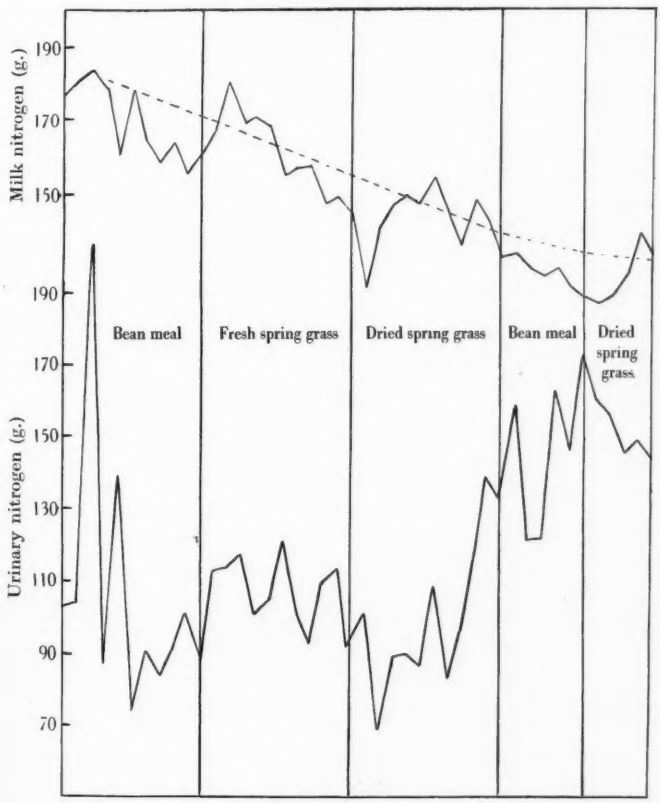


Fig. 7. (Cow 6.)

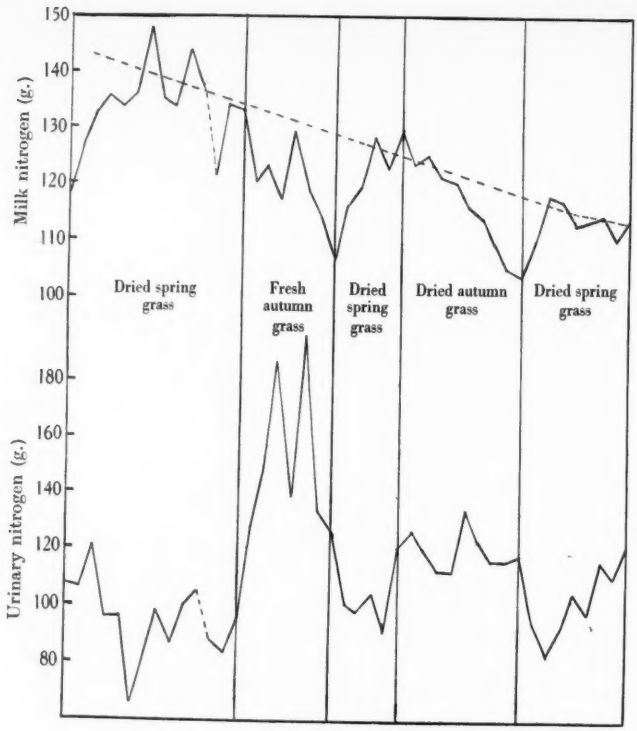


Fig. 8. (Cow 7.)

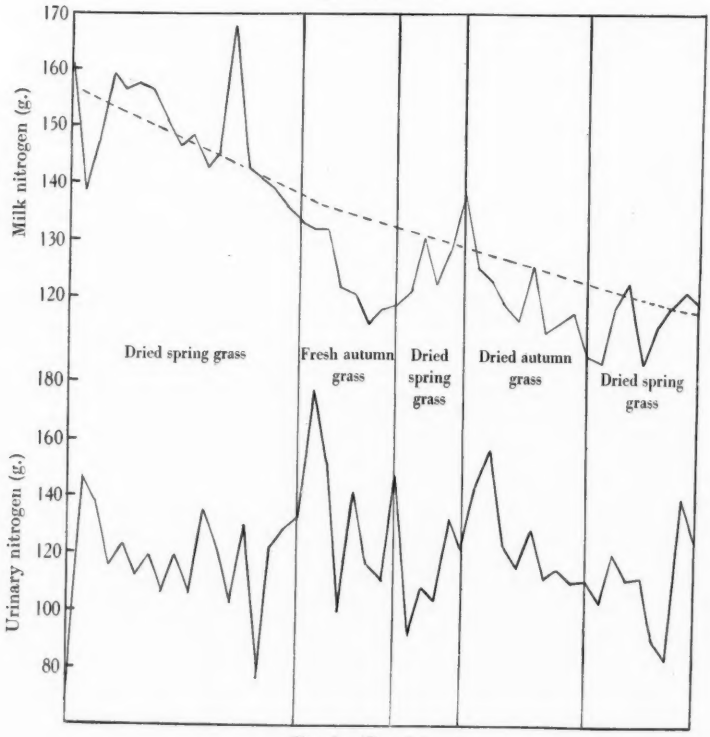


Fig. 9. (Cow 8.)

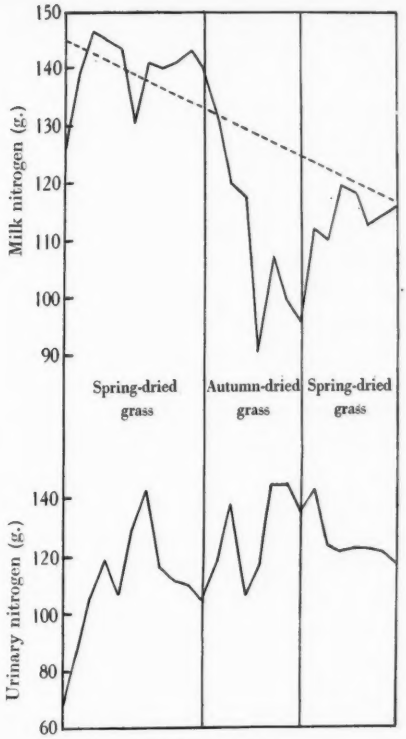


Fig. 10. (Cow 9.)

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619.2:618.19-002.5 + 616.999.61:612.664.36

# 133. INFLUENCE OF MASTITIS AND OF *BRUCELLA ABORTUS* INFECTION UPON THE MILK YIELD OF COWS

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"Milk yield is one of the most variable characters known. The standard deviation is a fourth to a fifth of the mean yield of the product, and the range for different individuals is about three-fourths the mean value on either side of this mean" (1). While milk yield is largely influenced by heredity, "environment" also plays a part, and among the factors which contribute to "environment" is disease. It is in fact well understood by veterinarians and becoming more widely recognized among owners of dairy stock that disease is one of the main causes of poor milk yield. In this respect the principal diseases are Johne's disease, contagious abortion, and mastitis, and there can be little doubt that the last-named is responsible for the greatest aggregate loss. The main object of the present work was to measure the loss due to mastitis and to determine whether it was significant in the statistical sense. The effect of abortion, however, also necessarily arises, and, therefore, the opportunity is taken to review the relevant literature on both diseases.

The statistical assessment of the loss from disease presents inherent difficulties owing to the numerous factors which influence the milk yield of cows. Corrections have to be made in the first place for age (as judged by the number of calvings), length of dry period, service period (interval between calving and next effective service) and month of calving. Comparisons of yield can only be made with animals of the same breed living under the same conditions of animal husbandry and being milked by the same system—by hand or machine, as the case may be. After all these conditions have been satisfied, it has to be remembered that other diseases may have a bearing on the issue. Thus, when the influence of mastitis on yield is being considered, the animals must be free from abortion, Johne's disease and tuberculosis. Finally, there are practical obstacles arising through sale, death or sporadic illness of animals whose milk records are desired. In view of the above difficulties it is not strange that in the past no exact and at the same time sufficiently extensive observations have been made as to the effect of disease on milk yield.

[1] Of the Medical Research Council's Statistical Staff.

## LITERATURE

### A. *Effect of abortion on milk yield*

It is accepted that cows which abort have shortened lactation periods and so have low total milk yields, also that those aborting early in gestation give less milk than those aborting a more mature foetus. It is not clearly established whether cows infected with *Br. abortus* but which do not abort give less milk than is normal. Presumably this may be so if one or more quarters of the udder are infected, since the work of Runnells & Huddleson(2), Tweed(3), Bang & Bendixen(4) and Niermann(5) has shown that *Br. abortus* may produce udder disturbances in the absence of mastitis streptococci.

The influence of *Br. abortus* infection has been studied at a number of the Experiment Stations in the United States.

Hooper(6), working with the herd of the Kentucky Experiment Station, examined the milk yields of six cows which aborted 131–183 days after service, of six cows which aborted 216–254 days after service and of six cows which completed the full gestation period of 280 days. The total average yields during 7 months prior to the month of parturition and during 7 months subsequent to the month of parturition were as follows. The average yield of each animal for the month before and after that of parturition is also shown.

| | Total average yield (lb.) | | Average yield (lb.) for month | |
|---|---|---|---|---|
| | Before parturition | After parturition | Before parturition | After parturition |
| Early aborters | 4288 | 3298 | 516 | 524 |
| Late aborters | 3650 | 4306 | 411 | 683 |
| Normal calvers | 3248 | 5949 | 281 | 1052 |

Hooper concludes that an abortion early in pregnancy stimulates the milk flow only slightly, a late abortion stimulates it considerably, but far less than a normal calving. In the above series, following an early abortion the decreased yield over the 7 months' period works out at 44 per cent., and following a late abortion 27 per cent. It is not said, however, that corrections were made, nor whether the cows compared were similar as regards age and in other respects.

Simms & Miller(7) in one herd found that during 4 years the average yield per cow in an abortion-infected group was about 30 per cent. less than in an abortion-free group, though no mention is made of correction factors. The data are as follows:

| Year | Cows | Non-reactors | Reactors | Normal calvers | Abortions | Average milk production (lb.) Non-reactors | Average milk production (lb.) Reactors | Diff. | Percentage reduction in yield |
|---|---|---|---|---|---|---|---|---|---|
| 1919 | 54 | 13 | 41 | 41 | 14 | 6693 | 4964 | 1729 | 25·8 |
| 1920 | 52 | 16 | 36 | 35 | 12 | 5704 | 4506 | 1198 | 21·0 |
| 1921 | 64 | 24 | 40 | 44 | 12 | 7976 | 5616 | 2360 | 29·5 |
| 1922 | 79 | 31 | 48 | 50 | 10 | 8542 | 4710 | 3830 | 44·8 |

In *Bulletin* 232 of the Oregon State Agricultural College(8), reference is made to the loss of milk due to abortion as observed in the College herd. A group of eight half-sisters, the progeny of the same bull, gave normal first calvings, and the yield at this calving was used as a basis for estimating their production at maturity. Six of the eight animals subsequently became infected and only one of the six reached the estimated normal production. On the average, the six animals were short of the estimated normal by 84 lb. butterfat, while the two which did not become infected exceeded the average by 38 and 79 lb. butterfat respectively. It is to be assumed that the six infected animals completed normal gestation periods.

White *et al.*(9) made observations over a number of years in the Connecticut Agricultural College Herd. During the years 1914–24 inclusive records were available for forty-five cows reacting to the agglutination test and thirty-eight non-reactors, the animals being closely comparable as regards average age, average years in milk per cow, average time between calvings, and number of calvings per cow. The data may be shown as follows:

| Group | No. | Cow years | Average years in milk | Average age | No. of lactations | Average time between calvings months | Calvings per cow | Total milk yield lb. | Average yield per lactation lb. | Diff. lb. |
|---|---|---|---|---|---|---|---|---|---|---|
| Reacting | 45 | 176 | 3·91 | 4 y. 8 m. | 129 | 13·3 | 2·86 | 995,228 | 7715 | 1600 |
| Non-reacting | 38 | 151 | 3·97 | 4 y. 10 m. | 108 | 13·8 | 2·84 | 1,006,061 | 9315 | |

The animals belonged to four different breeds, distributed as follows:

| Group | No. | Holstein | Cow years | Ayrshire | Cow years | Guernsey | Cow years | Jersey | Cow years |
|---|---|---|---|---|---|---|---|---|---|
| Reacting | 45 | 12 | 40 | 6 | 33 | 9 | 30 | 18 | 73 |
| Non-reacting | 38 | 15 | 70 | 11 | 31 | 5 | 24 | 7 | 26 |

Apparently during the 11 years' period there were thirty-seven premature calvings in the reacting group and five in the non-reacting group.

The group of reacting cows covering 176 cow years yielded 10,833 lb. less milk than the non-reacting group comprising a total of 151 cow years. The loss per lactation, therefore, amounted on an average to 1600 lb. milk, or 17 per cent. Several factors tended to make this loss a minimal one. Thus, some reacting cows which did not abort at the first pregnancy and which were later found to be inferior animals were later sold promptly if they calved prematurely. With such animals the yield following abortion was not used, whereas that following the normal calving was included. Again, the reacting group included a few cows which did not become reactors until middle life and a few that did not remain permanently infected. Finally, the results were influenced by one exceptional Ayrshire cow which, although a reactor, was in milk throughout the 11 years and did not abort. The influence of premature calving upon the yield is also shown by the following data for twenty-seven of the animals, the milk flow being corrected to full age, using the factors given

by Turner *et al.*(10). A special correction was also introduced for those cows which were entered in the United States Advanced Registry.

Twenty-seven calvings, before the 265th day; Average yield 6800 lb.
Same cows, normal periods ,, 8161 lb.]

There is thus a decrease due to abortion of 1361 lb. or a percentage decrease of 16·6. Thirteen of the twenty-seven calvings took place before the 215th day, and with these the percentage decrease was 22·2.

Fritz & Barnes(11) in Pennsylvania made observations in tubercle-free herds belonging to state institutions. The relevant information for three of these may be set out as follows. No mention is made of the use of correction factors.

| Herd | Category | No. of cows | Cow years | Total milk yield lb. | Average yield per year lb. | Diff. | Per-centage decrease |
|---|---|---|---|---|---|---|---|
| B | Non-reacting | 104 | 198 | 2,602,415 | 13,143 | | |
| | Reacting | 60 | 96 | 906,027 | 9,438 | 3705 | 28 |
| D | Non-reacting | 88* | 147 | 906,216 | 6,165 | | |
| | Reacting | 40 | 70 | 360,775 | 5,153 | 1012 | 16 |
| F | Non-reacting | 17* | 34 | 257,465 | 7,573 | | |
| | Reacting | 20 | 37 | 218,176 | 5,897 | 1676 | 22 |
| B | Non-reacting | 18† | 26 | — | 13,012 | | |
| | Reacting | 18† | 26 | — | 7,516 | 5496 | 42 |

* One animal in each of these groups aborted.

† The same eighteen cows are recorded before and after reacting to the abortion test. After reacting, there were eleven abortions in seven of the eighteen cows.

The results with the eighteen animals of herd B, to which specific reference is made, are of particular interest. Apart from these, it is not clear how many abortions occurred among the reacting animals.

Graham & Thorp(12) in Illinois record that following twenty-eight normal calvings the average milk yield for a 10 months' lactation period amounted to 9740 lb., whereas following eleven abortions the average yield was 7612 lb., i.e. a difference of 2128 lb. or a percentage decrease of 21·8. These calvings and abortions occurred in sixteen animals of "comparable milking quality" over a period of 4 years. Further details are not given in this semi-popular bulletin.

Rich(13) consulted the records of the University of Minnesota herd for the period 1900–29. The records, which included those from 115 cows which aborted, showed that the average yearly butterfat production was 266 lb., while with the same cows after normal calving the production was 324 lb. The difference of 58 lb., equivalent to 1450 lb. of milk containing 4 per cent. butterfat, represents a decreased yield of 18 per cent. ascribed to abortion. The records of a few reactors to the agglutination test, which had not aborted, were also compared with those of a similar number of non-reactors, the progeny of the same sires, and kept under the same conditions. The results are of interest, but unfortunately, as the author says, the groups were too small to furnish significant results. Thus, twelve non-reactors in the University

herd gave an average butterfat yield of 336 lb., while the average yield of the twelve reactors was 312 lb., a difference of 7·1 per cent. in favour of the non-reacting groups. In a branch herd a comparison was made between the yield from nine lactations in positive cows and the yield from a similar number of lactations in a negative group. With the latter the average figure for butterfat production was 388 lb., compared with 342 lb. for the reactors, a difference of 11·8 per cent. in favour of the non-reactors. In Rich's work, production records represent a complete lactation if this was less than 365 days; if the lactation period exceeded 1 year the records for the first 365 days were used. The yields were corrected to a "mature equivalent" basis, using tables furnished by the United States Department of Agriculture.

The work referred to above certainly gives some idea of the loss of milk which has been put down to abortion, but it is not clear in most cases that the milk yields were comparable, and there is no indication that the influence of mastitis was taken into account.

### B. *Effect of mastitis on milk yield*

Gross estimates have been made of the loss of milk caused through mastitis. For instance, Klimmer (quoted by Seelemann(14)) considered that the reduction in yield owing to mastitis might be put at 10 per cent.

Only two references have been found to work in which an attempt was made to measure the fall in yield. Seelemann(15) compared the yield of infected quarters with the corresponding healthy quarters of the opposite side. In the first place, the yield of normal quarters was measured; as found by others, the fore-quarters give less milk than the hind-quarters, the proportion according to Seelemann being 4 to 5·1. Records for twelve cows showing infection in one or more quarters are given to illustrate the effect of mastitis, but as they only concern one milking or very few milkings for each cow, they are not reproduced here. According to the figures given, infected quarters usually gave less than the estimated normal milk yield, but in a few instances the converse was the case. Seelemann emphasizes that quarters merely affected in a latent or subclinical way show a diminished yield, while occasional infected cows give quite a high yield over several lactations, the result possibly of a compensatory process.

Shaw & Beam(16) have also compared the yield of infected quarters with those of the opposite side. Eighty-six cows infected in one quarter were examined and the results are set out as follows:

| Quarter | State | No. | Average milk production lb. | Average butterfat production lb. |
|---|---|---|---|---|
| Fore | Non-infected | 41 | 2·3 | 0·11 |
| | Infected | 41 | 1·5 | 0·07 |
| Hind | Non-infected | 45 | 3·8 | 0·14 |
| | Infected | 45 | 2·6 | 0·08 |

In this series of cows, after allowing for the maximum variation found in the milk and butterfat production of non-infected quarters, mastitis was

considered to lower milk production by approximately 21 per cent. and butterfat production by 25 per cent.

## The herds examined

We have attempted to overcome the difficulties referred to in the introduction by working with large herds in which many or all of the cows were of the same breed, were free from tuberculosis and, as far as possible, from any disease other than mastitis which might affect the yield. Observations of sufficiently wide scope to merit presentation were carried out in two self-contained herds, the constitution of which at the beginning was as follows:

Herd I. 149 cows: 130 Friesians, 10 Shorthorns and 9 Guernseys.

Herd J. 203 cows: 143 Ayrshires, 46 Friesians, 7 Guernseys and 7 Shorthorns.

In herd J the animals were arranged in three and more recently in four groups on separate premises, but, as they have been under the same management and kept under the same conditions throughout, and as there has been a certain amount of interchange, the groups have been treated as one herd for the purpose of this work. With herd I the observations cover the milk-recording years 1929–31 and with herd J 1930–4, cows in their first, second, third and fourth or more lactations being included. In herd I milking has always been by machine, followed by hand-stripping; in herd J it has been by hand, except that in one group from May 1932 onwards a machine was used. This alteration in the system of milking in herd J in all probability does not invalidate the results for reasons which will be mentioned in the discussion.

### *History of disease*

*Tuberculosis.* Both herds are grade A tuberculin-tested; occasional animals which reacted to the periodic tuberculin tests were, therefore, at once removed.

*Contagious abortion.* At the beginning both herds contained reactors to the blood agglutination test, and serious attempts have been made to eliminate the disease by isolation and eventual disposal of infected stock. In herd I out of 195 cows tested in June 1931, 111 were negative, 57 were positive and 27 gave doubtful reactions. In July 1932 the milking herd consisted of 51 animals of which 49 were negative and 2 positive. In herd J the first complete test was carried out in November 1931, when 61 reactors were found. This number has been gradually reduced by sale, so that at the end of 1932, 1933, 1934 and 1935 respectively there were present 43, 30, 15 and 9 reactors.

*Johne's disease.* In herd I there was a little trouble from Johne's disease in 1930; but the animals affected were not used for the purpose of this paper. Herd J has always been apparently free from Johne's disease.

*Mastitis.* In both herds mastitis has been a serious trouble, and the incidence of infection was determined by making a bacteriological examination of milk samples from individual cows. Thus, in herd I in 1929, of 158 cows

examined 87 (55 per cent.) were found to be infected in at least one quarter of the udder; in herd J between November 1931 and June 1932, 83 cows out of 203 were found to be infected, an incidence of 40 per cent. These figures refer to the common chronic and contagious form of mastitis caused by *Streptococcus agalactiae*, and in both herds this was the predominant form of the disease. From time to time, however, there have appeared cases of mastitis due to other organisms, viz. *C. pyogenes* and streptococci other than *Str. agalactiae* (Minett(17)). In both herds efforts have been made to eradicate the contagious form of the disease by systematically milking infected cows last. In herd I this policy has been entirely successful, partly owing to the extensive sale of infected cows, so that since the end of 1932 this kind of infection has been absent. In herd J the disease is well under control, and the number of infected cows still present in the herd in October 1935 was 34 out of a total cow population of 162. Practically all the infected animals are now confined to separate premises. Eradication from herd J was assisted by the formation early in 1933 of a group built up exclusively from first-calving heifers, kept apart from other animals. An account of the control work carried out in these two herds has been published recently (Stableforth *et al.*(18)).

In connexion with the foregoing work, samples of milk from the individual quarters of the udder were examined at intervals. Thus, samples were taken at intervals of about 3 months in the case of animals of the "healthy" group that were in milk; with infected animals which were retained in the herd, samples were taken at intervals of 6–12 months or sometimes longer. It should be pointed out that in the ordinary course of eradication work, cows once found to be infected are not re-examined because as a rule infection does not clear up spontaneously. With cows in the healthy group, however, frequent examinations are necessary in order to guard against new infections. Technical details of the examination have been given elsewhere (Minett(17)). In brief, milk samples from individual quarters were taken as cleanly as possible, usually at the morning milking, and sent by train to the Research Institute where they were examined at once. The reaction of each milk sample was estimated by the use of brom-cresol-purple papers, the milk was then centrifuged in approximately 10 ml. amounts, and after the quantity and characters of any deposit had been recorded the upper 9 ml. were rejected and the residue used for cultural examination. For this purpose ox-blood agar containing crystal violet in a final concentration of 1 in 200,000 was used, two poured plates being put up from the contents of each centrifuge tube, one with half the deposit undiluted and the second from remaining deposit diluted at 1 in 10. The plates were examined after 36–48 hours at 37° C. and the result recorded. Deep colonies of *Str. agalactiae* are frequently surrounded by a narrow clear zone of haemolysis, and when colonies are numerous, verification of their nature is unnecessary once some experience has been acquired. When haemolytic colonies are few or when the streptococci are non-haemolytic, verification is necessary. The use of crystal violet is of great advantage in inhibiting the

growth of most milk organisms other than streptococci, so that with the use of this dye the reading of plates becomes a relatively simple task.

### Milk yield records of herds I and J

Milk yields were taken each year from the registers kept in accordance with the requirements of the Milk-Recording Society. Information as to dates of calving, length of dry period, service dates, etc., was also taken from the Register, supplemented where necessary by private communication with the owner. Records of whole lactations only were used. The cows were sorted into four categories by one author (F. C. M.) who at the time was unaware of the milk records. The categories were:

Cows free from both mastitis and abortion.

Cows not reacting to the abortion test but infected with mastitis.

Cows free from mastitis but reactors to the test for abortion.

Cows affected with mastitis and reacting to the abortion test.

For the sake of brevity the four categories are referred to below as the M− A−, M+ A−, M− A+, M+ A+ groups.

It was sometimes rather difficult to decide from the laboratory records whether a cow should be classed as mastitis-infected or not. In such cases the clinical history was taken into account. The aim was to include in the mastitis-healthy group cows whose laboratory and herd history in respect of *Str. agalactiae* was definitely clean. Cows whose milk contained haemolytic staphylococci or streptococci other than *Str. agalactiae* were, however, included in the healthy group, unless there was evidence that these organisms were causing marked and enduring disturbance. Cows were classed as "mastitis-infected" (*a*) when one quarter of the udder at least was definitely infected with *Str. agalactiae*, irrespective of the herd history, (*b*) when the milk was definitely abnormal at one or more examinations as regards reaction or centrifuge deposit, even although recognizably pathogenic bacteria were not demonstrable, but only if the owner could state the animal had suffered from udder trouble. Cows were excluded from consideration if the laboratory findings were as (*b*), but if the owner was unaware of any abnormalities in the udder or milk. Heifers calving with "blind quarters" were also excluded unless the owner could definitely state that the fault in the quarter had been associated with suppuration, in which case it was included as a case of mastitis. Exclusion of all doubtful cows has been rigorous, in fact about one-third of the cows on the milk registers had to be excluded from consideration because the data were insufficient or could not be satisfactorily interpreted. The correction factors introduced for standardizing the milk yields have been those advocated by Sanders (19), the method taking into account the month of calving, service period, age and dry period.

The results for cows of both herds are set out in Table I. The M− A− cows had an advantage over the other groups when all lactations were

considered together, although the difference exhibited by the M− A+ group was not statistically significant. The most unfavourable standardized yield was that of the M+ A+ group. The corrected average yield of this group was statistically in defect of that of the M+ A− group. The yields in order of lactation were affected in some cases by the small numbers on which the averages were based. For the first three lactations the M− A− cows did not show the definite advantage over the M+ A− and M+ A+ groups

Table I. *All cows.* 1930–4

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | All lactations | | | |
| Total lactations | 154 | 128 | 42 | 70 |
| Average yield (uncorrected) | 7627 | 7533 | 7201 | 7354 |
| Average yield (corrected) | 8803 ± 117 | 7849 ± 144 | 8137 ± 274 | 7118 ± 181 |
| Standard deviation | 2159 | 2420 | 2630 | 2244 |
| Range of yield (corrected) | 3241–16116 | 3536–19239 | 2055–14186 | 1541–11672 |
| Diff. between healthy and (2), (3) and (4) | — | 954 ± 185 | 666 ± 298 | 1685 ± 215 |
| | 1st lactation | | | |
| Total lactations | 84 | 21 | 25 | 7 |
| Average yield (uncorrected) | 7322 | 6602 | 6573 | 6896 |
| Average yield (corrected) | 9202 ± 141 | 8928 ± 392 | 7972 ± 359 | 8589 |
| Standard deviation | 1912 | 2663 | 2659 | 1735 |
| Range of yield (corrected) | 5245–16116 | 3536–13739 | 2055–13009 | 5594–11129 |
| Diff. between healthy and (2), (3) and (4) | — | 274 ± 417 | 1230 ± 386 | 613 |
| | 2nd lactation | | | |
| Total lactations | 40 | 35 | 3 | 12 |
| Average yield (uncorrected) | 7360 | 7111 | 6158 | 6017 |
| Average yield (corrected) | 8341 ± 242 | 8094 ± 306 | 7002 | 6486 ± 511 |
| Standard deviation | 2266 | 2684 | — | 2626 |
| Range of yield (corrected) | 3241–13430 | 3670–19239 | 5610–7066 | 1541–9848 |
| Diff. between healthy and (2), (3) and (4) | — | 247 ± 390 | 1339 | 1855 ± 565 |
| | 3rd lactation | | | |
| Total lactations | 12 | 20 | 2 | 12 |
| Average yield (uncorrected) | 7137 | 7702 | 10543 | 7837 |
| Average yield (corrected) | 7586 ± 376 | 8036 ± 345 | 10978 | 7542 ± 329 |
| Standard deviation | 1933 | 2286 | — | 1691 |
| Range of yield (corrected) | 5174–10053 | 4469–12885 | 5631–13416 | 4638–9468 |
| Diff. between healthy and (2), (3) and (4) | — | −450 ± 510 | −3392 | 44 ± 500 |
| | 4th lactation and over four lactations | | | |
| Total lactations | 18 | 52 | 12 | 39 |
| Average yield (uncorrected) | 9974 | 7843 | 8214 | 7699 |
| Average yield (corrected) | 8770 ± 418 | 7198 ± 133 | 8291 ± 494 | 6917 ± 239 |
| Standard deviation | 2628 | 1423 | 2536 | 2214 |
| Range of yield (corrected) | 7036–13920 | 3834–10969 | 4360–14186 | 3619–11672 |
| Diff. between healthy and (2), (3) and (4) | — | 1572 ± 439 | 479 ± 647 | 1853 ± 482 |

which they exhibited in the total lactations. The M− A+ group had a corrected yield statistically less than that of the M− A− cows for the first lactation, which also differed from the findings for all lactations.

Most of the animals examined belonged to two breeds, viz. Ayrshire and Friesian, and these breeds were, therefore, considered separately. The analysis for the Ayrshires (herd J) is set out in Table II. The differences between the M− A− cows and the other three groups were not so striking as those for

all cows. The defect in the average corrected yield of the M+ A− group, when compared with the M− A− cows, was statistically significant. The difference between the M− A− group and the M+ A+ group, although large, cannot be held to be significant. The difference between the M− A+ group and the M− A− animals was very small when compared with its

Table II. *Herd J. Ayrshires.* 1931–4

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | All lactations | | | |
| Total cows | 70 | 27 | 22 | 20 |
| Total lactations | 84 | 48 | 24* | 24 |
| Average yield (uncorrected) | 7376 | 7857 | 7552 | 8154 |
| Average yield (corrected) | 8250±141 | 7358±150 | 8101±344 | 7518±266 |
| Standard deviation | 1912 | 1540 | 2496 | 1932 |
| Range of yield (corrected) | 3241–13920 | 4216–12885 | 3126–14186 | 4620–11672 |
| Diff. between healthy and (2), (3) and (4) | — | 892±206 | 149±372 | 732±301 |
| | 1st lactation | | | |
| Total lactations | 42 | 4 | 9 | — |
| Average yield (uncorrected) | 6675 | 5941 | 7060 | — |
| Average yield (corrected) | 8522±119 | 7718 | 8189 | — |
| Standard deviation | 1141 | — | 2679 | — |
| Range of yield (corrected) | 5247–12648 | 6442–10489 | 3126–11995 | — |
| Diff. between healthy and (2), (3) and (4) | — | 804 | 333 | — |
| | 2nd lactation | | | |
| Total lactations | 20 | 5 | 2 | 1 |
| Average yield (uncorrected) | 6826 | 6676 | 5624 | 5934 |
| Average yield (corrected) | 7674±314 | 7377 | 6339 | 6681 |
| Standard deviation | 2084 | — | — | — |
| Range of yield (corrected) | 3241–11590 | 5780–9393 | 5611–7066 | — |
| Diff. between healthy and (2), (3) and (4) | — | 297 | 1335 | 993 |
| | 3rd lactation | | | |
| Total lactations | 7 | 4 | 1 | 2 |
| Average yield (uncorrected) | 6720 | 8621 | 7893 | 6530 |
| Average yield (corrected) | 6832 | 8897 | 8541 | 6009 |
| Standard deviation | 1695 | — | — | — |
| Range of yield (corrected) | 3924–9188 | 6627–12885 | — | 4638–7381 |
| Diff. between healthy and (2), (3) and (4) | — | −2065 | −1709 | 823 |
| | 4th lactation and over four lactations | | | |
| Total lactations | 15 | 35 | 12 | 21 |
| Average yield (uncorrected) | 10377 | 7947 | 8214 | 8415 |
| Average yield (corrected) | 8915±485 | 7138±178 | 8291±494 | 7701±287 |
| Standard deviation | 2786 | 1564 | 2536 | 1952 |
| Range of yield (corrected) | 6683–13920 | 4216–10969 | 4360–14186 | 4620–11672 |
| Diff. between healthy and (2), (3) and (4) | — | 1777±517 | 624±692 | 1214±564 |

* Three lactations followed abortion.

probable error. When the data were displayed in order of lactations the numbers in the subdivisions were too small for any valid conclusions to be drawn.

Tables III and IV show the results of the analysis for the Friesians belonging to herds J and I respectively. The M− A− cows showed a

considerable advantage over each of the other three groups, the difference being significant in each case. The definite lower yield of the M− A+ group exhibited by herd I was not shown in Tables I or II. The lowest yield was that of the M+ A+ groups, and the M+ A+ group in herd J had a significantly smaller yield than the M+ A− group. When the data were divided into order

Table III. *Herd J. Friesians.* 1931–4

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | All lactations | | | |
| Total cows | 19 | 14 | 2 | 15 |
| Total lactations | 21 | 24 | 2 | 20 |
| Average yield (uncorrected) | 8542 | 8174 | 7443 | 6878 |
| Average yield (corrected) | 10196 ±242 | 8208 ±279 | 9388 | 6694 ±392 |
| Standard deviation | 1647 | 2027 | — | 2598 |
| Range of yield (corrected) | 5818–12592 | 5168–12885 | 8878–9897 | 1541–10093 |
| Diff. between healthy and (2), (3) and (4) | — | 1988 ±369 | 808 | 3502 ±461 |
| | 1st lactation | | | |
| Total lactations | 18 | 1 | 2 | 2 |
| Average yield (uncorrected) | 8465 | 8875 | 7443 | 6767 |
| Average yield (corrected) | 10306 ±276 | 11147 | 9388 | 9433 |
| Standard deviation | 1733 | — | — | — |
| Range of yield (corrected) | 5818–12592 | — | 8878–9897 | 9003–9863 |
| Diff. between healthy and (2), (3) and (4) | — | −841 | 918 | 873 |
| | 2nd lactation | | | |
| Total lactations | 2 | 2 | — | 4 |
| Average yield (uncorrected) | 8384 | 8652 | — | 5450 |
| Average yield (corrected) | 9058 | 8639 | — | 5515 |
| Standard deviation | — | — | — | — |
| Range of yield (corrected) | 8524–9592 | 7137–10141 | — | 1541–9442 |
| Diff. between healthy and (2), (3) and (4) | — | 419 | — | 3543 |
| | 3rd lactation | | | |
| Total lactations | 1 | 7 | — | 3 |
| Average yield (uncorrected) | 10233 | 8297 | — | 9348 |
| Average yield (corrected) | 10468 | 8591 | — | 9506 |
| Standard deviation | — | 2378 | — | — |
| Range of yield (corrected) | — | 5631–12885 | — | 8697–10093 |
| Diff. between healthy and (2), (3) and (4) | — | 1877 | — | 962 |
| | 4th lactation and over four lactations | | | |
| Total lactations | — | 14 | — | 11 |
| Average yield (uncorrected) | — | 7994 | — | 6744 |
| Average yield (corrected) | — | 7745 ±356 | — | 5858 ±414 |
| Standard deviation | — | 1974 | — | 2035 |
| Range of yield (corrected) | — | 5168–10374 | — | 3169–9806 |
| Diff. between healthy and (2), (3) and (4) | — | — | — | — |

of lactation the numbers were too small in most groups for comparative purposes. For the first and second lactations the advantage possessed by the M− A− cows over the M+ A− group was not significant.

The observations in herds I and J, therefore, showed that by the use of Sanders's method of standardization mastitis had an adverse effect upon the milk yield, and that contagious abortion also definitely lowered the yield among the Friesians.

Table IV. *Herd I. Friesians.* 1930–1

| | Mastitis – Abortus – (1) | Mastitis + Abortus – (2) | Mastitis – Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | All lactations | | | |
| Total cows | 26 | 36 | 12 | 14 |
| Total lactations | 39 | 56 | 12 | 19 |
| Average yield (uncorrected) | 8006 | 6847 | 6431 | 7032 |
| Average yield (corrected) | 9718 ±243 | 8116 ±274 | 7702 ±630 | 7416 ±350 |
| Standard deviation | 2246 | 3042 | 3237 | 2260 |
| Range of yield (corrected) | 5613–16116 | 3536–19239 | 2055–13416 | 2439–11129 |
| Diff. between healthy and (2), (3) and (4) | — | 1602 ±366 | 2016 ±675 | 2302 ±426 |
| | 1st lactation | | | |
| Total lactations | 20 | 16 | 11 | 4 |
| Average yield (uncorrected) | 7851 | 6625 | 5816 | 6603 |
| Average yield (corrected) | 9948 ±376 | 9092 ±474 | 7182 ±582 | 8626 |
| Standard deviation | 2490 | 2810 | 2863 | 1992 |
| Range of yield (corrected) | 6411–16116 | 3536–13739 | 2055–13009 | 5594–11129 |
| Diff. between healthy and (2), (3) and (4) | — | 856 ±605 | 2766 ±693 | 1322 |
| | 2nd lactation | | | |
| Total lactations | 15 | 28 | — | 7 |
| Average yield (uncorrected) | 8196 | 7078 | — | 6353 |
| Average yield (corrected) | 9531 ±367 | 8148 ±428 | — | 7013 |
| Standard deviation | 2109 | 3359 | — | 2420 |
| Range of yield (corrected) | 5613–13431 | 3670–19239 | — | 2439–9848 |
| Diff. between healthy and (2), (3) and (4) | — | 1383 ±564 | — | 2518 |
| | 3rd lactation | | | |
| Total lactations | 3 | 9 | 1 | 6 |
| Average yield (uncorrected) | 7785 | 6830 | 13192 | 7149 |
| Average yield (corrected) | 9019 | 7222 | 13416 | 6958 |
| Standard deviation | 947 | 1656 | — | 1341 |
| Range of yield (corrected) | 7764–10053 | 4469–10180 | — | 5450–9468 |
| Diff. between healthy and (2), (3) and (4) | — | 1797 | –4397 | 2061 |
| | 4th lactation and over four lactations | | | |
| Total lactations | 1 | 3 | — | 2 |
| Average yield (uncorrected) | 8923 | 5915 | — | 9914 |
| Average yield (corrected) | 10030 | 5302 | – | 7785 |
| Standard deviation | — | — | – | — |
| Range of yield (corrected) | — | 3834–6914 | — | 4553–11017 |
| Diff. between healthy and (2), (3) and (4) | — | 4728 | — | 2245 |

## THE STANDARDIZATION OF MILK YIELDS

The question arose whether the standard used in the preceding work was a suitable one to apply to the data. Sanders's data consisted of nearly four thousand lactations arranged in nine classes comprising five different breeds. Less than half were pedigree cows. The number of lactations through which a cow lives was given as 3·27 in the Norfolk area and was stated to be less for the Penrith area. It may be noted that taking the present herds, I and $J_2$ together the average was 2·9. In the cases cited the percentage age distribution was as follows:

| Lactation | Sanders's herds: Norfolk | Sanders's herds: Penrith | Herds I and J |
|---|---|---|---|
| 1st | 23·5 | 34·3 | 34·6 |
| 2nd | 20·9 | 28·5 | 23·1 |
| 3rd | 17·6 | 18·1 | 11·8 |
| 4th | 13·6 | 10·3 | 6·4 |
| 5th | 9·6 | 5·1 | 8·0 |
| 6th | 6·1 | 1·9 | 8·8 |
| 7th | 4·2 | 0·9 | 5·1 |
| 8th | 2·3 | 0·7 | 1·6 |
| 9th | 1·2 | 0·15 | 0·5 |
| 10th | 0·5 | — | — |
| 11th | 0·3 | 0·05 | — |
| 12th | 0·1 | — | — |
| 13th | 0·06 | — | — |
| 14th | 0·02 | — | — |
| 15th | 0·02 | — | — |
| | 100·00 | 100·00 | 99·9 |

Thus, the two sets of data are rather similar, except that Sanders had a greater variety of breeds. There remained, however, an important point, namely, whether the standard used by Sanders was capable of differentiating satisfactorily between healthy cows and cows in an early state of disease. Sanders had no knowledge of the condition of the animals used for obtaining his standard, but any objection from this angle could be dismissed if the proportion of cows with mastitis and abortion was constant at each age. The actual distribution of disease in the herds together but separated as regards breed was as follows:

| Lactation | Ayrshires: M− A− | Ayrshires: M+ A− M− A+ M+ A+ | Friesians: M− A− | Friesians: M+ A− M− A+ M+ A+ |
|---|---|---|---|---|
| 1st | 42 | 13 | 38 | 36 |
| 2nd | 20 | 8 | 17 | 41 |
| 3rd | 7 | 7 | 4 | 26 |
| 4th | 3 | 10 | 1 | 10 |
| 5th | 5 | 15 | — | 10 |
| 6th | 4 | 23 | — | 6 |
| 7th | 3 | 14 | — | 2 |
| 8th | — | 4 | — | 2 |
| 9th | — | 2 | — | — |
| | 84 | 96 | 60 | 133 |

Since it is generally agreed that the proportion of cows affected with mastitis rises with age, it can be assumed that herds I and J are representative. Hence it follows that any standard based on non-examined herds must have been heavily weighted after the second lactation in favour of diseased animals. Only one-sixth of the above lactations from the third onwards related to healthy cows. It seemed most likely, therefore, that the use of Sanders's standard, in the particular problem under discussion, would tend to mask differences existing between healthy and infected cows.

It was of interest to compare the relation between milk yield and the other variables as found in herds I and J and as found by other workers. These correlations are set out in Table V. The number of healthy Friesians which

Table V. *Correlation coefficients*

## Table V. *Correlation coefficients*

| | $r_{ab}$ | $r_{ax}$ | $r_{ay}$ | $r_{az}$ | $r_{bx}$ | $r_{by}$ | $r_{bz}$ | $r_{xy}$ | $r_{xz}$ | $r_{yz}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **First calvers** | | | | | | | | | | |
| Ayrshires (M − A −) | — | — | — | — | — | — | — | $N=40$<br>$\cdot578 \pm \cdot071$ | $N=40$<br>$\cdot470 \pm \cdot083$ | $N=40$<br>$\cdot767 \pm \cdot044$ |
| Friesians (M − A −) | — | — | — | — | — | — | — | $N=78$<br>$\cdot672 \pm \cdot042$ | $N=78$<br>$\cdot296 \pm \cdot070$ | $N=78$<br>$\cdot647 \pm \cdot044$ |
| Ayrshires (M + A −, M − A + and M + A +) | — | — | — | — | — | — | — | $N=13$<br>$\cdot885 \pm \cdot040$ | $N=13$<br>$\cdot627 \pm \cdot114$ | $N=13$<br>$\cdot897 \pm \cdot036$ |
| Friesians (M + A −, M − A + and M + A +) | — | — | — | — | — | — | — | $N=35$<br>$\cdot495 \pm \cdot086$ | $N=35$<br>$\cdot146 \pm \cdot112$ | $N=35$<br>$\cdot688 \pm \cdot060$ |
| Sanders (19) | — | — | — | — | — | — | — | $N=879$†<br>$\cdot795 \pm \cdot008$ | $N=$†<br>$\cdot444 \pm \cdot018$ | — |
| **Other calvers** | | | | | | | | | | |
| Ayrshires (M − A −) | $N=41$<br>$-\cdot148 \pm \cdot103$ | $N=41$<br>$\cdot363 \pm \cdot091$ | $N=41$<br>$\cdot389 \pm \cdot089$ | $N=41$<br>$\cdot606 \pm \cdot067$ | $N=41$<br>$-\cdot099 \pm \cdot104$ | $N=41$<br>$-\cdot153 \pm \cdot103$ | $N=41$<br>$-\cdot144 \pm \cdot103$ | $N=41$<br>$\cdot834 \pm \cdot032$ | $N=41$<br>$\cdot609 \pm \cdot066$ | $N=41$<br>$\cdot673 \pm \cdot058$ |
| Friesians (M − A −) | $N=61$<br>$-\cdot165 \pm \cdot084$ | $N=61$<br>$-\cdot091 \pm \cdot086$ | $N=61$<br>$-\cdot033 \pm \cdot086$ | $N=61$<br>$\cdot136 \pm \cdot085$ | $N=61$<br>$-\cdot050 \pm \cdot086$ | $N=61$<br>$-\cdot244 \pm \cdot081$ | $N=61$<br>$-\cdot328 \pm \cdot077$ | $N=61$<br>$\cdot214 \pm \cdot082$ | $N=61$<br>$-\cdot073 \pm \cdot086$ | $N=61$<br>$\cdot357 \pm \cdot075$ |
| Ayrshires (M + A −, M − A + and M + A +) | $N=83$<br>$-\cdot177 \pm \cdot072$ | $N=83$<br>$\cdot132 \pm \cdot073$ | $N=83$<br>$\cdot087 \pm \cdot073$ | $N=83$<br>$\cdot182 \pm \cdot072$ | $N=83$<br>$-\cdot156 \pm \cdot072$ | $N=83$<br>$-\cdot189 \pm \cdot071$ | $N=83$<br>$-\cdot141 \pm \cdot073$ | $N=83$<br>$\cdot614 \pm \cdot046$ | $N=83$<br>$\cdot426 \pm \cdot061$ | $N=83$<br>$\cdot636 \pm \cdot044$ |
| Friesians (M + A −, M − A + and M + A +) | $N=93$<br>$\cdot050 \pm \cdot070$ | $N=93$<br>$\cdot056 \pm \cdot070$ | $N=93$<br>$-\cdot026 \pm \cdot070$ | $N=93$<br>$-\cdot033 \pm \cdot070$ | $N=93$<br>$-\cdot150 \pm \cdot068$ | $N=93$<br>$-\cdot018 \pm \cdot070$ | $N=93$<br>$-\cdot074 \pm \cdot070$ | $N=93$<br>$\cdot334 \pm \cdot062$ | $N=93$<br>$\cdot036 \pm \cdot070$ | $N=93$<br>$\cdot654 \pm \cdot040$ |
| Sanders (19) | *$N=2542$<br>$\cdot061 \pm \cdot013$ | *$N=2542$<br>$\cdot065 \pm \cdot013$ | — | — | *$N=2542$<br>$\cdot017 \pm \cdot013$ | — | — | $N=2542$<br>$\cdot758 \pm \cdot005$ | $N=$†<br>$\cdot407 \pm \cdot010$ | — |
| **All calvers** | | | | | | | | | | |
| Tuff (22) | — | — | — | *$N=561$<br>$\cdot537 \pm \cdot020$ | — | — | $N=59$<br>$\cdot103 \pm \cdot087$ | — | $N=83$<br>$\cdot186 \pm \cdot071$ | $N=83$<br>$\cdot318 \pm \cdot067$ |
| Tuff (22) | — | — | — | $N=44$<br>$\cdot471 \pm \cdot082$ | — | — | — | — | — | $N=576$<br>$\cdot414 \pm \cdot024$ |
| Gowen: Holstein-Friesian | — | — | — | *$N=2586$<br>$\cdot433 \pm \cdot011$ | — | — | — | — | — | — |
| Gowen (1) | — | — | — | — | — | — | — | — | $N=$†<br>$\cdot199 \pm \cdot016$ | — |
| Sanders (19): Mongrels | — | — | — | — | — | — | — | — | $N=$†<br>$\cdot419 \pm \cdot014$ | — |
| Red Polls | — | — | — | — | — | — | — | — | $N=$†<br>$\cdot350 \pm \cdot023$ | — |
| Lincoln Reds | — | — | — | — | — | — | — | — | $N=$†<br>$\cdot425 \pm \cdot025$ | — |
| Friesians | — | — | — | — | — | — | — | — | $N=$†<br>$\cdot410 \pm \cdot029$ | — |

$a$ = age from number of calves. $b$ = dry period. $x$ = service period. $y$ = lactation period. $z$ = milk yield. * $a$ = actual age.

† Where value of $N$ is omitted, the number of cows was not given.

had had two or more calves was small, and as the milk records of herd I, since it had been freed from mastitis and abortion, were accessible, they were obtained with the hope of increasing the numbers in the later lactations. Unfortunately, in herd I the life of the Friesians was very short and no lactations beyond the third were available. Actually there were forty cows with the first lactation, thirty-two with the second and seven with the third lactation. These records were added to the other healthy Friesians of herds I and J to find the relation between milk yield and the other variables. Among the Ayrshire first calvers the correlations were lower in the M– A– group than in the other three groups, but the reverse was found with the Friesians. Of the sixteen correlation coefficients that involved dry period among animals of herds I and J which had had more than one calf, all were negative with one exception, but the correlation was invariably very small, and only two were statistically significant. These were the two involving lactation period and milk yield among the healthy Friesians. There was a great contrast in the results obtained with healthy Ayrshires and healthy Friesians when cows which had had more than one calf were considered. With the Ayrshires all correlations that did not involve dry period were significant, but with the Friesians only the correlation between milk yield and lactation period was significant. The correlation between service period and milk yield with the Friesians was negligible, but in the case of Sanders's data this was significant and fairly large. Thus, the results obtained with the healthy and diseased groups indicated that a general standard was not the best one to use in the present investigation.

An attempt has been made to form a standard for the Ayrshires on the basis of the healthy cows. The dry period was disregarded, since we have seen from Table V that dry period had only a small correlation with the other variables and was, therefore, useless for the purpose of prediction. The number of lactations available was too small to subdivide into month of calving, so this variable was ignored. All the cows belonged to the one herd, viz. herd J, so that variation in feeding and management did not arise. A simple equation was fitted, by the method of least squares, to milk yield in terms of number of lactations, service period and lactation period. The equation obtained was:

$$\text{Yield in lb.} = 716{\cdot}267\ (\text{lactation}) + 3{\cdot}0982\ (\text{service period in days}) + 16{\cdot}7785\ (\text{lactation period in days}) + 143{\cdot}9117.$$

The standard chosen was the second lactation, a service period of 100 days and a lactation period of 320 days, these values being the means of the variables in round numbers. For this standard the theoretical yield by the equation was 7255. The method of standardizing was first to obtain a standardizing factor by calculating from the equation a theoretical yield and dividing this into the standard yield. The actual yield multiplied by the standardizing factor gave the standardized yield. An example to illustrate this method is, a cow gave a yield of 5934 in her second lactation period and

had a service period of 60 days and a lactation period of 255 days. Her calculated yield from the equation is 6041, the standardizing factor is 7255/6041 = 1·2010 and the standardized yield is 5934 × 1·2010 = 7127. The results obtained by this method and by Sanders's standard are shown in Table VI. It will be seen that the differences between the M− A− group and the other three groups was emphasized more by this method than by Sanders's method. The M+ A− group was the only one which had a statistically significant lower yield than the healthy cows by Sanders's method, but both M+ A− and M+ A+ groups were significantly worse than the M− A− cows when judged by the healthy Ayrshire standard, and the difference between the M− A− group and the M− A+ group approached significance.

The data for the Friesians were found to show defects when the construction of a simple standard was attempted. In the first place, there were no healthy lactations beyond the third, and extrapolation up to the eighth lactation, necessary to standardize the infected animals, was unjustified. We have seen from Table V that the correlations with milk yield were insignificant with the exception of the period of lactation, which was small. These small correlation values showed that a simple prediction formula could not be expected to give good results and the data were not extensive enough to warrant anything more elaborate than a straight line. Since it was of interest to make some estimate of the yield of the infected cows on the basis of the healthy animals, a similar equation to that fitted in the case of the Ayrshires was found. Only the lactations of herd I, including the seventy-nine additional lactations, were used. The equation was:

$$\text{Yield in lb.} = 684{\cdot}7752\ (\text{lactation}) + 5{\cdot}7524\ (\text{service period in days}) + 22{\cdot}7612\ (\text{lactation period in days}) + 831{\cdot}5926.$$

The standard chosen was the second lactation, a service period of 110 days and a lactation period of 300 days giving a theoretical yield of 8397 lb. The results obtained by the two methods of standardization for both herds, for the first three lactations, are shown in Table VII. The yields of the three infected groups of herd I were significantly less than the healthy cows by the new standard as worked out for Friesians. This standard gave a larger statistical difference for the M+ A− and M− A+ groups than did Sanders's method, but for the M+ A+ group the difference was greater by Sanders's method. The numbers in herd J were too small for the usual test of significance. The standardized yield by Sanders's method of the M+ A+ group was significantly less than that of the M− A− group when the "$t$" test was employed, but the M− A+ group and both the M+ A− and M+ A+ groups by the Friesian standard were not definitely significant.

Tables VI and VII confirm the conclusions, arrived at from Tables I–IV, that in herds I and J mastitis had a definitely adverse effect on the milk yield and that contagious abortion also lowered the milk yield. With the Ayrshires the M+ A− and M+ A+ groups had a yield of 10·8 and 8·9 per cent. less

Table VI. *Ayrshires. All lactations*

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| Total lactations | 84 | 48 | 24 | 24 |
| Average yield (uncorrected) | 7376 | 7857 | 7552 | 8154 |
| Sanders's standard: | | | | |
| Average corrected yield | 8250 ± 141 | 7358 ± 150 | 8101 ± 344 | 7518 ± 266 |
| Standard deviation | 1912 | 1540 | 2496 | 1932 |
| Diff. between healthy and (2), (3) and (4) | — | 892 ± 206 | 149 ± 372 | 732 ± 301 |
| Diff./P.E. | — | 4·3 | 0·4 | 2·4 |
| Ayrshire standard: | | | | |
| Average corrected yield | 7243 ± 95 | 6041 ± 136 | 6576 ± 225 | 6252 ± 225 |
| Standard deviation | 1264 | 1383 | 1631 | 1636 |
| Diff. between healthy and (2), (3) and (4) | — | 1202 ± 166 | 667 ± 244 | 991 ± 244 |
| Diff./P.E. | — | 7·2 | 2·7 | 4·1 |

*Sanders's standard*

| | | |
|---|---|---|
| Corrections employed: | | |
| Month of calving | ... | Standard, March and September approximately |
| Service period | ... | ,, 85 days |
| Age | ... | ,, Sixth lactation |
| Dry period | ... | ,, 40 days (second calvers and others separately corrected) |

*Ayrshire standard*

| | | |
|---|---|---|
| Service period | ... | Standard, 100 days |
| Lactation period | ... | ,, 320 days |
| Age | ... | ,, Second lactation |

Table VII. *Friesians. First to third lactation*

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | Herd I | | | |
| Total lactations | 38 | 52 | 11 | 15 |
| Average yield (uncorrected) | 7987 | 6927 | 6519 | 6853 |
| Sanders's standard: | | | | |
| Average corrected yield | 9705 ± 251 | 8314 ± 261 | 7706 ± 688 | 7627 ± 316 |
| Standard deviation | 2296 | 2795 | 3383 | 1813 |
| Diff. between healthy and (2), (3) and (4) | — | 1391 ± 362 | 1999 ± 732 | 2078 ± 404 |
| Diff./P.E. | — | 3·8 | 2·7 | 5·1 |
| Friesian standard: | | | | |
| Average corrected yield | 8155 ± 188 | 7090 ± 159 | 6370 ± 400 | 6937 ± 347 |
| Standard deviation | 1715 | 1703 | 1967 | 1991 |
| Diff. between healthy and (2), (3) and (4) | — | 1065 ± 246 | 1785 ± 442 | 1218 ± 395 |
| Diff./P.E. | — | 4·3 | 4·0 | 3·1 |
| | Herd J | | | |
| Total lactations | 21 | 10 | 2 | 9 |
| Average yield (uncorrected) | 8542 | 8426 | 7443 | 7042 |
| Sanders's standard: | | | | |
| Average corrected yield | 10195 ± 243 | 8856 | 9388 | 7716 |
| Standard deviation | 1653 | 2235 | — | 2835 |
| Diff. between healthy and (2), (3) and (4) | — | 1339 | 807 | 2479 |
| Friesian standard: | | | | |
| Average corrected yield | 8187 ± 164 | 7453 | 7096 | 7127 |
| Standard deviation | 1113 | 1448 | — | 1490 |
| Diff. between healthy and (2), (3) and (4) | — | 734 | 1091 | 1060 |

*Sanders's standard*

| | | |
|---|---|---|
| Corrections employed: | | |
| Month of calving | ... | Standard, March and September approximately |
| Service period | ... | ,, 85 days |
| Age | ... | ,, Sixth lactation |
| Dry period | ... | ,, 40 days (second calvers and others separately corrected) |

*Friesian standard*

| | | |
|---|---|---|
| Service period | ... | Standard, 110 days |
| Lactation period | ... | ,, 300 days |
| Age | ... | ,, Second lactation |

than the M− A− cows when judged by Sanders's standard, and a defect of 16·6 and 13·7 per cent. when the new (Ayrshire) standard was used. With the Friesians (first three lactations) in herd I the M+ A− and M+ A+ groups had a loss of 14·3 and 21·4 per cent. by Sanders's standard and 13·1 and 14·9 per cent. by the new (Friesian) standard.

## Discussion

In the foregoing work the records of 373 lactations have been examined, viz. 180 in Ayrshires (herd J) and 193 in Friesians (67 in herd J and 126 in herd I). The number of cows involved was: 139 Ayrshires and 138 Friesians (50 in herd J and 88 in herd I).

One of the main difficulties has been to obtain a means of expressing adequately the difference between the yield of healthy and of diseased animals. Sanders's method of standardization was the only one readily available, but as Sanders could not take into account the disease factor his method was obviously not very suitable for the present purpose. The provision of a standard based on the yield of cows known to be free from the common contagious diseases of cattle has been attempted, but the small number of healthy cows which had passed more than one or two lactations has made impossible for the time being the laying down of a standard applicable to all lactations. Nevertheless, even when such a standard was applied on the necessarily limited scale, differences between healthy and diseased cows which are without significance by Sanders's standard become definitely significant.

Before discussing the extent of the lowered milk yield in herds I and J, certain points should be considered. In both herds the mastitis infection followed the usual insidious and slowly progressive type, and there was nothing of the nature of an acute outbreak which would tend to make the reduction in yield unusually severe. Comparatively few cows had entirely lost a quarter as a result of disease. Moreover, the extent of disease in the individual animals was not unduly great, the average number of diseased quarters for each infected cow working out at 2·3. This figure closely agrees with that given by Seelemann (15), who examined milk samples bacteriologically from 1416 quarters of 394 affected cows and found infection in 843 (59·5 per cent.) quarters, or 2·4 quarters per cow. Also, the average number of infected quarters per cow in the two mastitis groups of each herd was nearly the same, viz. 2·1 and 2·1 in herd I and 2·7 and 2·5 in herd J.

A further point concerns the method of milking. As mentioned earlier, in herd J a proportion of the cows were for a time milked by machine. It might conceivably be argued that the reduction in yield in the mastitis groups was due not to disease but to the fact that some cows were milked by machine, since, as pointed out by Dahlberg (20) and by Bartlett & Huthnance (21), machine-milked cows may give rather lower yields than cows milked by hand—quite possibly as a result of inefficient handling of the machine. With

our data, however, such a consideration scarcely arises, since in the mastitis group machine milking was adopted with only six cows over thirty-three lactation months as compared with twenty-six cows over 228 lactation months in the healthy group. The possibility of some reduction in yield due to machine milking was, therefore, far greater in the healthy than in the infected group.

It was of interest to see how our figures for percentage loss of milk due to mastitis or abortion or both would compare with those given for the loss ascribed by other investigators to contagious abortion. The data are summarized in Table VIII. Unfortunately, a satisfactory comparison cannot be made because in most of the American investigations it must be presumed that correction factors were not used and mastitis apparently was not taken into consideration. If one excludes the high figures found for two series of animals in the American work—and in one of the two series it is stated that the figures refer to abortions very early in pregnancy—the mean percentage difference in milk yield works out at 21, with extremes of 16 and 29. In two series where corrections were made the percentage differences were 16·6 and 18. In our work, when all cows were considered and abortion infection only was included, the percentage difference in yield was 7·6, but it reached 20·7 with the Friesians of herd I, although these animals did not abort. Among the Ayrshires in herd J abortion infection was apparently of insignificant importance in lowering the milk yield. Mastitis alone caused a percentage reduction of 10·8 when all cows were considered; the reduction was the same with the Ayrshires, but with the Friesians of herds I and J the respective figures were 16·5 and 19·5. When the combined effect of mastitis and abortion was considered, the percentage difference with all cows was 19·1; with the Ayrshires it was 8·9, and with the Friesians of herds I and J the respective figures were 23·7 and 34·3. It will be noticed that the reduction in yield was much more serious among the Friesians than among the Ayrshires. This is not due to the Friesians being older on the average than the Ayrshires, though in this connexion it should be remembered that young abortion-reactors tend to abort more frequently than older cows and would, therefore, be expected to show a greater loss. Actually, 68 per cent. of the Friesians in herds I and J were in the first or second lactation, while only 16 per cent. of them had reached four or more lactations. In herd J the Friesians with four or more calves were four times as numerous as similar cows in herd I. By comparison, 46 per cent. of the Ayrshires were in the first or second lactation and a similar proportion had had four or more calves. The differing reduction of yield in the two breeds is not, therefore, an age effect, nor is it due to actual abortions occurring. Apparently the explanation is that in a high-yielding breed the milk-secreting tissue is destroyed in relatively higher measure when it is invaded by pathogenic bacteria than is the case with breeds giving a more moderate yield. The American figures, therefore, by comparison with ours are high. It is quite possible that the abortion-reactors in the United States investigations were older animals and thus were affected to a greater degree

with mastitis in a subclinical form. In other words, the American figures may represent the loss in milk not only from abortion but also in part from mastitis.

Table VIII. *Percentage reduction in milk yield ascribed to mastitis or* Brucella abortus *infection or to both*

| Authors | Category of cow | No. of lactations | | Average milk yield lb. | Percentage decrease | Remarks |
|---|---|---|---|---|---|---|
| Hooper (6) | (1) Normal calvings | 6 | | 5949* | (1) and (2) 44 | ? uncorrected yields. Three groups of 6 cows each |
| | (2) Early abortions | 6 | (6) | 3298* | (1) and (3) 27 | |
| | (3) Late abortions | 6 | (6) | 4306* | | |
| Simms & Miller (7) | Abortion non-reactors | 13 | | 6693 | 25·8 | ? uncorrected yields |
| | ,, reactors | 41 | (14) | 4964 | | |
| | Abortion non-reactors | 16 | | 5704 | 21 | |
| | ,, reactors | 36 | (12) | 4506 | | |
| | Abortion non-reactors | 24 | | 7976 | 29·5 | |
| | ,, reactors | 40 | (12) | 5616 | | |
| | Abortion non-reactors | 31 | | 8542 | 44·8 | |
| | ,, reactors | 48 | (10) | 4710 | | |
| White *et al.* (9) | Abortion non-reactors | 108 | (5) | 9315 | 17 | Cows were comparable |
| | ,, reactors | 129 | (37)† | 7715 | | |
| Fritz & Barnes (11) | Abortion non-reactors | 198 | | 13143 | 28 | ? uncorrected yields |
| | ,, reactors | 96 | | 9438 | | |
| | Abortion non-reactors | 147 | | 6165 | 16 | |
| | ,, reactors | 70 | | 5153 | | |
| | Abortion non-reactors | 34 | | 7573 | 22 | |
| | ,, reactors | 37 | | 5897 | | |
| Graham & Thorp (12) | Normal calvings | 28 | | 9740 | 21·8 | Uncorrected yields. The 39 calvings were in 16 animals said to be comparable |
| | Abortions | 11 | (11) | 7612 | | |
| Rich (13) | Normal calvings | ? 115 | | 8100 | 18‡ | Corrected yields. Results of the same cows are compared |
| | Abortions | ? 115 | (115) | 6650 | | |
| Minett & Martin | Ayrshire and Friesian (herds J and I) | | | | | |
| | (1) M− A− | 154 | | 8803 | (1) and (2) 10·8 | Effect of mastitis |
| | (2) M+ A− | 128 | | 7849 | (1) and (3) 7·6 | Effect of abortion |
| | (3) M− A+ | 42 | (3) | 8137 | (1) and (4) 19·1 | Effect of mastitis and abortion |
| | (4) M+ A+ | 70 | | 7118 | | |
| | Ayrshire (herd J) | | | | | |
| | (1) M− A− | 84 | | 8250 | (1) and (2) 10·8 | Effect of mastitis |
| | (2) M+ A− | 48 | | 7358 | (1) and (3) 1·8 | Effect of abortion |
| | (3) M− A+ | 24 | (3) | 8101 | (1) and (4) 8·9 | Effect of mastitis and abortion |
| | (4) M+ A+ | 24 | | 7518 | | |
| | Friesian (herd J) | | | | | |
| | (1) M− A− | 21 | | 10196 | (1) and (2) 19·5 | Effect of mastitis |
| | (2) M+ A− | 24 | | 8208 | (1) and (3) 7·9 | Effect of abortion |
| | (3) M− A+ | 2 | | 9388 | (1) and (4) 34·3 | Effect of mastitis and abortion |
| | (4) M+ A+ | 20 | | 6694 | | |
| | Friesian (herd J) | | | | | |
| | (1) M− A− | 39 | | 9718 | (1) and (2) 16·5 | Effect of mastitis |
| | (2) M+ A− | 56 | | 8116 | (1) and (3) 20·7 | Effect of abortion |
| | (3) M− A+ | 12 | | 7702 | (1) and (4) 23·7 | Effect of mastitis and abortion |
| | (4) M+ A+ | 19 | | 7416 | | |

Figures in brackets in column 3 indicate the number of actual abortions.

* During 7 months subsequent to that of parturition.

† Lactations following twenty-seven of these abortions were compared with those following normal calvings of the same cows. The average percentage fall in yield (corrected figures) due to abortion was found to be 16·6.

‡ Percentage differences of 7·1 and 11·8 in favour of non-reactors are also quoted by Rich in two small groups of cows in which the reactors did not abort.

A question to be considered is whether reduction in yield in the infected cows was due to shortening of the lactation periods or to decrease in the quantity of milk given from day to day. The average number of days the cows

in the different groups were recorded is set out in Table IX. It is seen that there was no difference in the average length of the lactation periods among the different groups of Friesians in herd I, or of Ayrshires, but that in herd J the Friesians belonging to the M+ A− and M+ A+ groups had significantly shorter lactation periods than the healthy cows although they did not abort. The data shown in Table IX, when considered in conjunction with the results given in Tables II and IV, add emphasis to the fact that both mastitis and *Br. abortus* infection may cause significant reduction in yield without shortening the lactation period. This point is of particular interest and importance in infections due to *Br. abortus*. A final point to be noted from Table IX is that the healthy Friesians in herd I had significantly shorter lactation periods than those of herd J, and this, it will be observed, is reflected in the lower yield of the healthy Friesians in herd I compared with those in herd J (Tables III and IV).

Table IX. *Lactation periods—all lactations*

| | Mastitis − Abortus − (1) | Mastitis + Abortus − (2) | Mastitis − Abortus + (3) | Mastitis + Abortus + (4) |
|---|---|---|---|---|
| | Ayrshires | | | |
| Herd J: | | | | |
| No. of lactations | 84 | 48 | 24 | 24 |
| Mean duration of lactation (days) | 317 ±4·8 | 311 ±5·8 | 309 ±12·7 | 299 ± 7·5 |
| Standard deviation | 65 | 60 | 92 | 54 |
| Diff. between (1) and (2), (3) and (4) | — | 6 ±7·6 | 8 ±13·6 | 18 ± 8·9 |
| | Friesians | | | |
| Herd J: | | | | |
| No. of lactations | 21 | 24 | 2 | 20 |
| Mean duration of lactation (days) | 344 ±5·6 | 311 ±6·5 | 350 | 281 ±10·9 |
| Standard deviation | 38 | 47 | — | 72 |
| Diff. between (1) and (2), (3) and (4) | — | 33 ±8·6 | −6 | 63 ±12·2 |
| Herd I: | | | | |
| No. of lactations | 39 | 56 | 12 | 19 |
| Mean duration of lactation (days) | 301 ±7·0 | 288 ±6·2 | 329 ±20·3 | 299 ± 9·2 |
| Standard deviation | 65 | 69 | 104 | 59 |
| Diff. between (1) and (2), (3) and (4) | — | 13 ±9·4 | −28 ±21·5 | 2 ±11·5 |

The results obtained in this work should be extended when a suitable standard applicable to the different breeds has been formulated. As they stand, however, the results are of interest from the economic point of view. If a loss of milk, found by Sanders's method to amount on an average to 89 gallons per lactation in the Ayrshire breed and to 160 gallons in Friesians, may be expected to accompany *Streptococcus* mastitis in its subclinical form, the aggregate loss to the dairying industry from this cause alone must be tremendous. And this, of course, is not the only factor to be considered. Allowance has to be made for the excessive herd wastage and the depreciation in market value of affected animals, and, finally, there is the effect of the disease upon milk quality. The results presented also go to show that the increased cost of milk production caused through mastitis is raised still further by infection with contagious abortion, even though the cow does not actually abort.

Summary

The influence of mastitis and of *Br. abortus* infection, separately and combined, has been observed in two tuberculosis-free herds during the years 1929–31 and 1930–4 respectively. Actual abortions in these herds were few, but many cows were affected with *Streptococcus* mastitis in a subclinical form. The results, comprising the examination of 180 lactations in Ayrshires and 193 in Friesians, have been considered in relation to the results of others who have investigated the influence of *Br. abortus* infection on the yield.

When all the lactations of all cows were considered, the average reduction in yield per lactation was 954 lb. due to mastitis and 1685 lb. due to mastitis and abortion combined. Among the Ayrshires of one herd the average reduction due to mastitis was 892 lb. Among the Friesians forming the second herd, the average reduction was 1602 lb. due to mastitis, 2016 lb. due to abortion and 2302 lb. due to both diseases combined. In all these cases the defect in yield was of statistical significance, the records being standardized by Sanders's method. This method, however, is less suitable for the present purpose than one based on the milk yield of cows known to be free from the common contagious diseases of cattle. When this more suitable method is used, differences in yield between healthy and diseased cows, insignificant by Sanders's method, become more prominent or attain significance.

In terms of percentage fall in yield by comparison with that for healthy cows, the results may be expressed as follows. Among the Ayrshires mastitis reduced the corrected yield on an average by 10·8 per cent.; among the Friesians the percentage loss was higher, averaging 16·5 and 19·5 respectively in the two herds. Among the Friesians forming one herd the percentage reduction in corrected yield due to *Br. abortus* infection averaged 20·7, in spite of the fact that these animals did not abort.

Acknowledgement is made to Dr Hilda M. Woods who started the statistical part of the work in herd I. Our thanks are also due to Prof. Major Greenwood, F.R.S., for permitting the statistical part of this work to be carried out in his department.

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# 134. THE TITRATION OF MILK AND WHEY AS A MEANS OF ESTIMATING THE COLLOIDAL CALCIUM PHOSPHATE OF MILK

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(With 4 Figures)

While the ultimate composition of the mineral matter of milk has been known for many years, the precise mode of combination of the separate acid and basic constituents is a subject which still requires elucidation. Apart from its great academic interest, the solution of the problem becomes urgent in view of the rapid development of the dairy industry, and the increasing tendency to subject milk to processes which are known to disturb the delicate balance of its mineral constituents. The problem is complicated by the fact that some are found in both the soluble and colloidal phases, and may also exist in both organic and inorganic combination. Thus Na, K, Cl are almost exclusively confined to the soluble phase, while Ca, Mg, $P_2O_5$ occur in the soluble and colloidal states; and further, the elements Ca and P are found in combination with caseinogen.

Although it is generally agreed that the major part of the colloidal inorganic phase consists of calcium phosphate, opinions are divided as to whether it is the di- or tricalcium salt. It is clearly a matter of utmost importance to discover which form is present in milk since, if dicalcium phosphate is present, it is necessary to allocate a high proportion of calcium to caseinogen, as opposed to a lower proportion if the tricalcium salt is present. The behaviour of these two alternative complexes cannot be assumed identical.

The distribution of phosphorus is very much more complex than that of calcium because its soluble compounds are not entirely inorganic, but include considerable amounts of phosphoric esters (1, 2, 3). Van Slyke & Bosworth (4), Palmer (5), Grimmer (6), and Wright (7), maintain that the colloidal inorganic phosphate is in the form of the dicalcium salt; Porcher (8) and Soldner (9) consider that it is composed of a mixture of di- and tricalcium phosphates; while Lehman (10), Lindet (11), and Pyne (12) contend that tricalcium phosphate accounts for the major portion of the colloidal phosphate of milk. A study of these works also reveals a great diversity of opinion on the question of the supposed association of the colloid phosphate with caseinogen. Some of these workers (10, 11, 12), as well as Hammarsten (13) and Gyorgy (14), consider that the colloid phosphate is combined to caseinogen, most probably through the calcium salt of this protein; while others (9) either reject the association entirely or concede only a protective role to the protein (4, 8). Richmond (15) advances a formula for the caseinogen phosphate complex: $Ca_3Na_3H$ casein $+ 2Ca_3P_2O_8$.

Pyne (12) investigated the problem, using the well-known effect of neutral potassium oxalate on the titration of milk. This effect was used by Van Slyke & Bosworth (16) to give what they considered to be the true titration value of milk. They claimed that phosphate solutions containing calcium cannot be titrated accurately because, as soon as neutral dicalcium phosphate is formed, it undergoes hydrolysis which leads ultimately to the precipitation of tricalcium phosphate and the liberation of phosphoric acid:

$$\begin{aligned} &CaHPO_4 + 2H_2O = Ca(OH)_2 + H_3PO_4, \\ &2CaHPO_4 + Ca(OH)_2 = Ca_3P_2O_8 + 2H_2O \\ \hline &3CaHPO_4 = Ca_3P_2O_8 + H_3PO_4 \end{aligned}.$$

Thus, the titration of acid phosphate solutions in the presence of calcium gives results which are in excess of the true acidity by a quantity which, for the sake of clarity in the following treatment, will be termed the overrun ($o$). By adding to milk 2 per cent. of a saturated solution of potassium oxalate, these authors claimed that a true titration value was obtained, since the disturbing influence of calcium had been removed through its precipitation as oxalate. Pyne criticizes this view, and demonstrates that potassium oxalate also reacts with the colloidal phosphate to develop alkalinity. A case is quoted in which the addition of 4 per cent. saturated potassium oxalate to milk actually made the sample alkaline. A comparison of the titration values of milk with the whey produced therefrom showed that the addition of increasing amounts of oxalate to milk reduced its titration value to a minimum at 3–4 per cent. oxalate, whereas with whey a minimum value was reached at about 0·75 per cent. oxalate. This difference of oxalate effect between milk and whey is attributed by Pyne and Ryan (17) to the following reaction between colloidal tricalcium phosphate and potassium oxalate:

$$Ca_3P_2O_8 + 3K_2 \text{ oxalate} = 3Ca \text{ oxalate} + 2K_3PO_4.$$

Experiments with calcium-caseinogen-phosphate complexes confirmed this view. It is also apparent that above 0·75 per cent. of potassium oxalate, it is the colloidal tricalcium phosphate which causes the further reduction of the titration value; so that the results obtained by the titration of milk, to which 2 per cent. potassium oxalate has been added, cannot possess the significance attributed to them by Van Slyke & Bosworth. The work of Pyne indicates that most, if not all, of the colloidal inorganic phosphate of milk is tricalcium phosphate, which in his view is attached to the calcium salt of caseinogen as follows:

$$= (COO)_2Ca + Ca_3P_2O_8 = (COO—Ca—PO_4{=}Ca)_2.$$

By estimating the alkalinity produced by the reaction between potassium oxalate and tricalcium phosphate, it should be possible to determine the latter in milk. This may be done by titration of milk and its whey, with and without the addition of 4 per cent. saturated neutral potassium oxalate solution.

The ordinary titration value of milk ($M_1$) may be considered as the sum

of three factors, namely, caseinogen acidity ($c$), true serum acidity ($s$), and the overrun previously described ($o$).

$$M_1 = c + s + o,$$

After adding oxalate, the overrun is eliminated, but a new factor is introduced, namely the alkalinity ($X$) developed from tricalcium phosphate. Thus the titration value with 4 per cent. oxalate $(M_2) = (c+s) - X$. With whey

$$W_1 \text{ (without oxalate)} = s + o \quad \text{and} \quad W_2 \text{ (with oxalate)} = s.$$

Assuming the values ($c$) and ($s$) to be unaffected by the oxalate,

$$M_1 - M_2 = o + X, \quad W_1 - W_2 = o$$

and therefore

$$(M_1 - M_2) - (W_1 - W_2) = X.$$

Using the relationship $Ca_3P_2O_8 \equiv 2K_3PO_4 \equiv 2$ litres of $N$ acid, the value $X$ may be converted to grams of tricalcium phosphate.

The present paper represents an attempt to examine the possibilities of this method for estimating the colloidal tricalcium phosphate of milk:

(1) by comparing it with the value obtained for colloidal inorganic $P_2O_5$ obtained from estimations of total inorganic $P_2O_5$ in milk and whey;

(2) by observing the effect of the development of lactic acid in milk on its tricalcium phosphate as determined by the titration method; and

(3) by a comparison of the actual increase of CaO and $P_2O_5$ in the whey during such development of acidity, with those calculated from the disappearance of tricalcium phosphate as revealed by the titration figures.

## I. Evidence furnished by inorganic $P_2O_5$ estimations on milk and whey

### *Experimental*

*Titrations.* The titration of oxalated samples presented no difficulties, the end-point with phenolphthalein being very well defined. But with untreated samples the end-point was vague, and it was found essential to work with a very definite pink colour standard. Several standards were tried and the most satisfactory was found to be a 0·25 per cent. aqueous solution of rosaniline hydrochloride. Duplicate titrations agreed closely when carried to the colour of 25 ml. of milk to which 0·14 ml. of colour solution had been added. Titrations were made in duplicate on 10 ml. portions in small wide-necked flasks. Six drops of phenolphthalein indicator were used and the alkali solution was $N/10$ strength.

*Preparation of the whey.* 100 ml. of milk at 30° C. were coagulated with 0·7 ml. rennet extract. The curd was cut with a stainless steel knife and the whey allowed to drain for a few minutes before filtering by decantation through a Whatman No. 40 paper. To expedite filtering, a battery of four separate filters was used, and as soon as any of the papers showed signs of clogging they were immediately renewed. In this way about 50 ml. of whey were obtained in a reasonably short time.

*Inorganic* $P_2O_5$ *determinations.* To 1 volume of milk or whey contained in a suitable measuring flask, 4 volumes of 10 per cent. trichloracetic acid solution were added with constant rotation of the flask, the liquid being finally made up to the mark. After shaking and allowing to stand for an hour, the liquid was filtered through a Whatman No. 12 fluted paper. The filtrates were usually quite clear, but occasionally it was necessary to filter again through a No. 44 paper. Of the filtrate, 10 ml. were made up to 250 ml. with distilled water in a measuring flask. The colorimetric method used was that of Kuttner & Lichtenstein(18), in which stannous chloride was employed as the reducing agent. Five ml. of diluted sample were measured into a small beaker, 4 ml. of molybdate reagent added (1 vol. 10*N* $H_2SO_4$, 1 vol. 7·5 per cent. sodium molybdate, 2 vol. distilled water), and then 1 ml. stannous chloride reagent (10 g. $SnCl_2$ in 25 ml. HCl diluted as required to 1 in 200). On mixing, the blue colour developed rapidly and was compared with a 0·030 mg. P standard for milk samples and 0·015 mg. P for wheys. These measurements were made in a dark room, using a Hellige immersion colorimeter with lamp attachment. Duplicate estimations were invariably made, and it was found that the method gave excellent results.

*Method of calculation.* All estimations were reduced to a weight basis. With whey, a further adjustment was necessary to bring results from a whey percentage to a milk percentage. Neglecting the small amount of fat escaping in the whey, and assuming that most of the soluble protein appears in the whey, the factor 100−(per cent. Fat+[per cent. Protein−0·4]) was chosen to relate the whey figures to the original milk. To check the accuracy of this factor, several samples of milk were allowed to sour, after having determined the total calcium content. After filtration, estimations of calcium on the sour serum were made with the following results:

| % CaO | | % CaO in serum times factor |
|---|---|---|
| Milk | Serum | 100 − (fat + [protein − 0·4]) |
| 0·1798 | 0·1926 | 0·1791 |
| 0·1778 | 0·1894 | 0·1774 |
| 0·1724 | 0·1905 | 0·1768 |
| 0·1799 | 0·1944 | 0·1810 |
| 0·1680 | 0·1787 | 0·1674 |

It was therefore concluded that the use of this factor was justified within the limits of experimental error involved.

As regards the titration figures, the corrections did not make any appreciable difference except with the acid samples, where they were of considerable magnitude. Taking $M_1$, $M_2$, $W_1$, and $W_2$ as the titration values for 10 ml. of milk and whey, the effect of the corrections are shown in the following table.

| | Fresh milk | | | Acid milk | | |
|---|---|---|---|---|---|---|
| | $M_1 - M_2$ | $W_1 - W_2$ | Difference $N/10$ alkali $\equiv Ca_3P_2O_8$ | $M_1 - M_2$ | $W_1 - W_2$ | Difference $N/10$ alkali $\equiv Ca_3P_2O_8$ |
| | 1·49 | 0·38 | 1·11 | 1·57 | 1·34 | 0·23 |
| Corrected | 1·46 | 0·35 | 1·11 | 1·54 | 1·22 | 0·32 |
| | 1·34 | 0·29 | 1·05 | 1·41 | 1·03 | 0·38 |
| Corrected | 1·31 | 0·27 | 1·04 | 1·38 | 0·95 | 0·43 |
| | 1·35 | 0·34 | 1·01 | 1·42 | 1·49 | −0·07 |
| Corrected | 1·32 | 0·31 | 1·01 | 1·39 | 1·37 | +0·02 |

*Colloidal inorganic* $P_2O_5$. This was obtained as the difference between the percentage total inorganic phosphate in milk and that for the whey corrected by the factor already described.

*Results*

The following table gives the results of thirteen samples of morning milk from the College herd of 40–50 Dairy Shorthorns. All the samples were stored at 45° F. until required in the laboratory.

| Sample no. | % inorganic $P_2O_5$ in milk *A* | Inorganic $P_2O_5$ in whey (% times factor) *B* | Colloidal inorganic $P_2O_5$ *A* − *B* | $P_2O_5$ in $Ca_3P_2O_8$ (by titration) | Difference |
|---|---|---|---|---|---|
| 1 | 0·1613 | 0·0866 | 0·0747 | 0·0704 | +0·0043 |
| 2 | 0·1687 | 0·0878 | 0·0809 | 0·0733 | +0·0076 |
| 3 | 0·1728 | 0·0868 | 0·0860 | 0·0814 | +0·0046 |
| 4 | 0·1591 | 0·0780 | 0·0811 | 0·0764 | +0·0047 |
| 5 | 0·1888 | 0·1000 | 0·0888 | 0·0798 | +0·0090 |
| 6 | 0·1729 | 0·0890 | 0·0839 | 0·0770 | +0·0069 |
| 7 | 0·1680 | 0·0929 | 0·0751 | 0·0700 | +0·0051 |
| 8 | 0·1643 | 0·0837 | 0·0806 | 0·0728 | +0·0078 |
| 9 | 0·1681 | 0·0919 | 0·0762 | 0·0719 | +0·0043 |
| 10 | 0·1616 | 0·0861 | 0·0755 | 0·0743 | +0·0008 |
| 11 | 0·1666 | 0·0864 | 0·0802 | 0·0732 | +0·0070 |
| 12 | 0·1616 | 0·0866 | 0·0750 | 0·0693 | +0·0057 |
| 13 | 0·1496 | 0·0774 | 0·0722 | 0·0692 | +0·0030 |

As the titration estimate of $Ca_3P_2O_8$ is the result of four separate values, it is clear that, even when each is done in duplicate, the method cannot hope for an accuracy much greater than ±0·05 ml. *N*/10 alkali or ±0·00355 per cent. $P_2O_5$. The agreement is therefore very striking, and it would appear that $Ca_3P_2O_8$ accounts for almost all the colloidal inorganic phosphate of milk. The observed discrepancy may be experimental, but from its constancy of sign the writer is of the opinion that it is a significant value, accounted for in all probability by colloidal magnesium phosphate. Estimations of magnesium in milk and whey on two different samples gave a colloidal magnesium content of 0·0035 and 0·0040 per cent. respectively. If present as $Mg_2H_2P_2O_8$, it would account for 0·0108 per cent. $P_2O_5$, while if as $Mg_3P_2O_8$ it would be equivalent to 0·0072 per cent. $P_2O_5$. Further support for the view that this discrepancy is significant is seen in Fig. 2, in which it is shown that more $P_2O_5$ actually passes into the whey during the development of acidity than is accounted for by the disappearance of colloidal $Ca_3P_2O_8$. In this case, when all the colloidal phosphate has been dissolved, the discrepancy is about 0·007 per cent. $P_2O_5$.

From an inspection of these figures, one may conclude that the titration method for the estimation of colloidal $Ca_3P_2O_8$ gives results which are sufficiently accurate to justify its use in the elucidation of problems concerned with the mineral balance of milk.

## II. The effect of developed lactic acid on the colloidal phosphate as determined by titration

### *Experimental*

The samples used for this experiment were again mixed morning milk from the College herd, kept in cold store until required (2–3 hours). They were inoculated at 60° F. with sufficient commercial starter to bring them to an acidity of 0·25–0·30 per cent. as lactic acid after 24 hours at 60° F. After this preliminary incubation period, the samples would rise from 0·30 per cent.

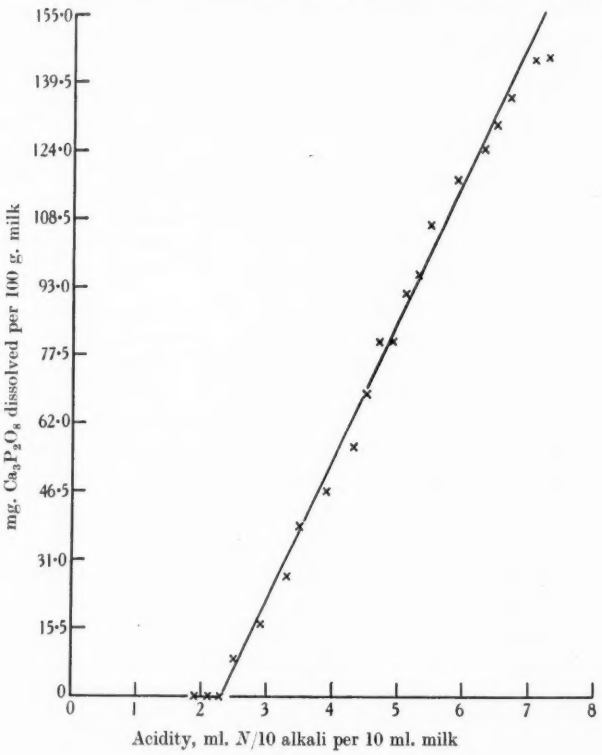


Fig. 1.

to coagulation point during about 7 hours when kept at 30°C. The titration values $M_1$, $M_2$, $W_1$, $W_2$, were determined immediately after inoculation, and also with rising acidity during the following day. All titrations were made in duplicate using the colour standard previously described. When acidity was developing rapidly, errors were likely to arise through a continued production of acid in the whey during filtration. To reduce this possibility to a minimum, the four 10 ml. portions of milk were measured, and a further 100 ml. were coagulated with 0·7 ml. of rennet extract. While coagulating,

the values $M_1$ and $M_2$ were determined on two of the milk samples. When all the whey had been decanted from the curd into the filters, the remaining two samples were similarly titrated and the two values of $M_1$ and $M_2$ were averaged. This method was considered preferable to any attempt to prevent acidity development during the filtering process.

*Results*

The composite results of twenty separate experiments are shown in Fig. 1. As the samples possessed different amounts of tricalcium phosphate, it was not possible to obtain a composite showing the amount of calcium phosphate remaining at different acidities. The graph therefore shows the actual solution of colloidal calcium phosphate in milk at various acidities. It is seen that the passage from the colloidal to the soluble phase takes place quite regularly, the graph being linear. On the average, solution is complete at about 7·5 ml. acidity (0·675 per cent. calculated as lactic acid). There were slight differences in the gradients of individual samples, due no doubt to the different ionic and molar concentrations of calcium, phosphate and citrate in the fresh milk sera. It appeared, therefore, that the titration method gave a true estimate of colloidal tricalcium phosphate in fresh and acid samples, but it was considered desirable to seek confirmation of this view by the following experiment.

## III. Evidence furnished by estimations of calcium and phosphorus in the whey obtained from milk at various acidities

*Experimental*

The experiment was conducted on the lines indicated above, with the addition of calcium and phosphorus estimations on the wheys.

*Calcium.* Duplicate portions of 25 ml. were treated with 100 ml. of 10 per cent. trichloracetic acid as previously described, and 100 ml. of the filtrate neutralized with ammonia and acidified with dilute acetic acid. Ammonium chloride was added and the calcium precipitated from hot solution with solid ammonium oxalate. After standing overnight, the precipitate was filtered, washed with hot water, and finally titrated with $N/20$ $KMnO_4$ in sulphuric acid solution. Results by this method were found to agree closely with the ashing method, and it has the advantage of speed.

*Total* $P_2O_5$. Two of the 10 ml. samples were transferred to Kjeldahl flasks after titration and treated with 10 ml. strong sulphuric acid. The samples were heated to drive off some of the water, and then oxidized with successive additions of strong nitric acid until clear. After dilution and boiling, the samples were neutralized with ammonia, acidified with strong nitric acid, and the total $P_2O_5$ estimated gravimetrically by the ammonium molybdate-magnesia mixture method.

*Results*

Fig. 2 is a composite of thirteen experiments showing the actual increase of total $P_2O_5$ in the whey compared with that calculated from the disappearance

of tricalcium phosphate as found by the titration method. It is evident that the two values agree closely, but here again the titration estimate is somewhat lower than the actual increase in whey $P_2O_5$, a circumstance already noted in the colloidal inorganic phosphate estimations. At an acidity of 7·5, the average acidity for complete solution, the discrepancy is 0·007 per cent., a difference of the same order as that previously noticed.

The increment of whey CaO shown on Fig. 3 indicates that the whey receives considerably more CaO than is accounted for by tricalcium phosphate. This is due to the removal of calcium from the calcium salt of caseinogen. The

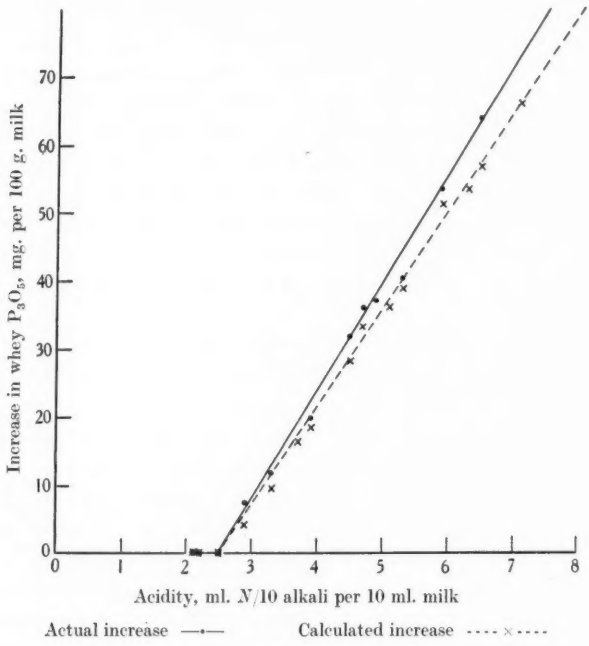


Fig. 2.

difference between the actual increment of CaO in the whey and that calculated from the disappearance of tricalcium phosphate gives, approximately, the amount of calcium removed from caseinogen as acidity increases, and it is on this basis that the lowest curve is constructed. This curve indicates that calcium from the protein salt is dissolved with greater difficulty than tricalcium phosphate. It is not easy to explain this difference if it is maintained that the phosphate is attached to the protein through the calcium of the protein salt, whereas if one concedes a separate existence, or at most an adsorbed existence for the colloidal phosphate, the difference in solubility might be expected. Further experiments are in progress which it is hoped will throw more light on the subject.

THE RELATIONSHIP BETWEEN THE ORDINARY TITRATION VALUES OF MILK AND WHEY, WITH AND WITHOUT ADDED OXALATE

Fig. 4, a composite of thirty-four experiments, shows the simple linear relationship which exists between the four titration values used in the estimation of tricalcium phosphate. The value $M_1 - W_1$ gives what might be called the curd acidity. It is interesting to note that as acidity develops in milk, this value also increases. As this is mainly the acidity contributed by the

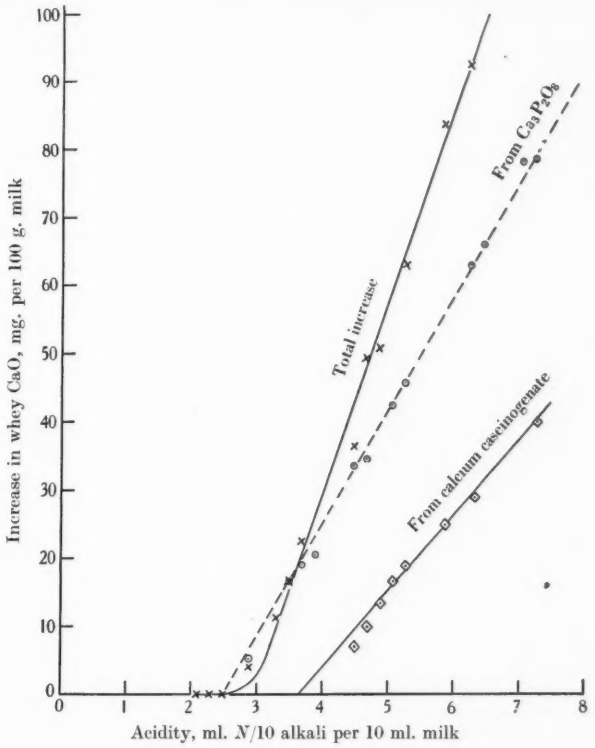


Fig. 3.

caseinogen to milk, the explanation of the observed increase of curd acidity is to be found in the removal of bases from the protein and their replacement by acid radicles or hydrogen ions. To obtain the true curd acidity it is necessary to bring the values $M_1$ and $W_1$ to a 10 g. of milk basis, using the corrections previously described. It is significant that the increment of the corrected curd acidity from 2·1 to 7·5 is 1·3 ml. $N/10$ alkali per 10 g. of milk. This corresponds to the removal of 0·0364 per cent. CaO from calcium caseinogenate. The average value for CaO attached to caseinogen was found to be 0·039 per cent.

INITIAL VALUES FOR MIXED MILK USED IN THESE EXPERIMENTS

The mean initial values and standard deviations of the samples were as follows:

| Determination | No. of samples | Mean | Standard deviation |
|---|---|---|---|
| $Ca_3P_2O_8$ | 33 | 0·1538 % | 0·0117 % |
| $M_1$ | 35 | 2·17 ml. | 0·15 ml. |
| $M_2$ | 35 | 0·80 ml. | 0·11 ml. |
| $W_1$ | 35 | 1·395 ml. | 0·11 ml. |
| $W_2$ | 34 | 1·01 ml. | 0·09 ml. |

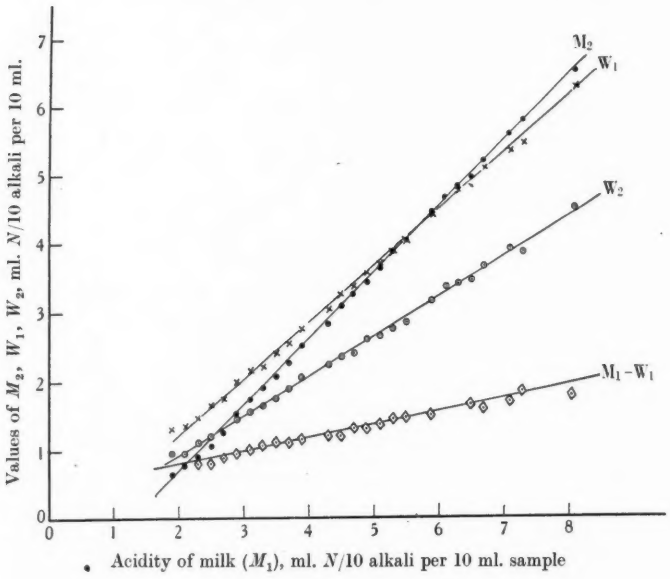


Fig. 4.

It has already been stated that the titration value of any sample of milk is composed of the caseinogen acidity ($c$), the serum acidity ($s$), and the overrun ($o$). Correcting the above mean figures to a basis of 10 ml. of milk, we obtain the values:

$$\text{Titration value of 10 ml. milk} = c + s + o,$$

$$\text{i.e. } 2{\cdot}17 = 0{\cdot}864 + 0{\cdot}943 + 0{\cdot}363.$$

Or, if the titration value be $x$,

$$\text{Caseinogen acidity} = 0{\cdot}399x,$$

$$\text{Serum acidity} = 0{\cdot}435x,$$

$$\text{Overrun} = 0{\cdot}166x.$$

These values apply to fresh samples only and are approximately in the ratio 0·4/0·4/0·2.

Summary

1. The titration method for the estimation of colloidal tricalcium phosphate in milk was found to agree closely with the determination of total colloidal inorganic $P_2O_5$.

2. The method was used to study the solution of tricalcium phosphate in milks of rising acidities.

3. Close agreement was obtained between the actual increase in total $P_2O_5$ in the wheys of souring samples and that calculated from the disappearance of tricalcium phosphate as found by the titration method.

4. The discrepancy between the actual increase in whey calcium in such cases and that calculated from the titration figures was attributed to the solution of calcium from the calcium salt of caseinogen. From figures so obtained it would appear that colloidal calcium phosphate dissolves with greater ease in milk than the calcium of the protein salt.

5. A linear relationship was found to exist between the ordinary titration value of milk and the three other values used in this estimation, and also between the curd acidity and milk acidity.

6. Average figures are given for the tricalcium phosphate content of the samples studied and for the various titration factors.

My thanks are due to Mr H. G. Robinson, M.Sc., Principal, Mr A. C. Burns, M.Sc., Head of the Chemical Department, and my colleagues in the Chemical, Dairy, and Farm Departments of the College, without whose co-operation this work could not have been undertaken.

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# 135. STUDIES ON THE CHEMISTRY OF CHEDDAR CHEESE MAKING

## IV. LACTOSE AND LACTIC ACID IN WHEY AND CURD; THE PRESENCE OF BOUND WATER IN CURD; THE EXISTENCE OF A DONNAN EQUILIBRIUM BETWEEN CURD AND WHEY; AND THE RATE OF PENETRATION OF SALT INTO CURD

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(With 6 Figures)

The significance of estimations of acidity in the whey as a means of following the course of fermentation of lactose by starter cultures in the process of manufacture of Cheddar cheese has already been discussed (1, 2) in relation to the mineral content of the whey. It has also been shown (3) that a mixed starter culture under conditions of continuous growth in milk gives an almost complete conversion of lactose into lactic acid. The work discussed in the present paper was designed to indicate the quantitative nature of the relationship between lactose and lactic acid in both curd and whey throughout the various stages of the cheese-making process.

The convention commonly used by cheese makers of expressing the acidity of the whey as "percentage lactic acid" (grams lactic acid per 100 ml. whey) is somewhat misleading and has frequently been responsible for the assumption that the figures represent actual concentrations of lactic acid. The acidity of fresh milk is actually a measure only of the buffering value of the proteins and mineral salts present, since, as is well known, fresh milk contains no lactic acid. When milk is allowed to sour through the growth of starter cultures, there is a close relationship between the rise in acidity and the increase in concentration of lactate (3). The production of lactic acid from lactose within the curd during the cheese-making process increases the mineral content of the wheys exuding from the curd (1, 10), and an absolute correspondence between acidity and lactate content of whey is not to be expected. The changes in acidity noted by the cheese maker are, however, frequently quite anomalous, in that at some of the later stages of the process the acidity may remain constant or show a marked fall, whereas other evidence, e.g. the feel of the curd, indicates that the acid production within the curd is proceeding steadily. Such decreases in acidity have been attributed to the increased fat content of the later wheys, but analyses of wheys have shown that the change in fat content is not sufficient to account for the decrease in acidity.

Van Slyke & Bosworth (4) estimated the lactose and lactic acid contents of curd at various stages during the cheese-making process, and of cheese at

various times after "hooping". They noted a rapid decrease in lactose content of the curd until a few hours after the cheese was hooped, and then a more gradual fall until within 2 weeks the lactose had almost completely disappeared. The lactic acid showed a corresponding rise in concentration, reaching a maximum about the time the last of the lactose was decomposed. Figures for the lactose and lactic acid contents of wheys were not obtained.

## Experimental

### *General procedure*

Cheese was made in the Institute factory in small vats (usually 40 gallons). Samplings of curd and whey were carried out simultaneously. Lactose and lactic acid were estimated on both curd and whey and also on the juice which had been expressed from the curd by Barthel's method (5). Moisture determinations were carried out on all samples, so that it was possible to relate all the analytical figures to the basis of parts per 100 parts of water.

### *Preparation of curd for analysis*

Samples of curd taken before the "drying" stage[1] were quickly dried on filter paper according to the method of Sammis *et al.* (6), and were then weighed out for analysis. Samples taken after "drying" were cut into strips and passed through a mincer; and the resultant finely divided product was well mixed before sampling.

### *Separation of juice*

Barthel (5) has shown that juice may be expressed from cheese if the finely divided product is first mixed with sand in suitable proportions. The present authors used a quartz sand which had been washed with acid and water and oven dried. Portions of the curd as prepared for analysis were mixed with an approximately equal weight of sand, enclosed in a bag of cheese cloth and subjected to pressures of up to 3 tons per sq. in. in an hydraulic press. A gradual application of the pressure was found to give better results than a quick increase to the maximum obtainable. The juice which, particularly at the later stages of the process, contained much fat, was whirled in large stoppered tubes in a steam Babcock centrifuge with the heating blast of steam turned on. The aqueous portion was then drawn off, filtered, and used for analysis.

### *Treatment of curd for lactose and lactic acid determination*

For estimation of the lactose and lactic acid it was necessary to dissolve and reprecipitate the protein of the curd. Two portions of 100 g. of prepared curd were weighed into 750 ml. Florence flasks and treated with 200 ml. of 0·4*N* sodium hydroxide solution. The flasks were shaken on a mechanical shaker for 30 min., and were then warmed in a water bath at 60° C. with

[1] The "drying" stage, referred to in this paper, is the New Zealand equivalent to "piling" in British cheese-making procedure.

frequent shaking until the contents had reached a temperature of about 50° C. After a vigorous shaking to emulsify the fat, the mixture was divided into two equal portions by weighing into two 250 ml. graduated flasks. One portion was used for the lactose and the other for the lactic acid estimation.

The quantity of alkali added was sufficient to produce a *p*H of 10–11 which appeared to prevent any further bacterial decomposition of the lactose. It was found also that the alkali itself was without effect on the lactose even with continued shaking for periods up to 3 hours. Heating of the mixture to temperatures much above 50° C. was avoided, as it caused some charring of the lactose.

*Estimation of lactose*

The method used for whey and for most of the juice samples has already been described(3). With curd it was necessary to modify the method of preparation of the sample as follows:

The alkaline emulsion corresponding to 50 g. of curd was treated gradually in a 250 ml. graduated flask with 20 ml. of 2*N* acetic acid, the theoretical quantity to neutralize the alkali added. The protein was precipitated with 30 ml. of 10 per cent. lead acetate solution, and the excess lead was removed with 20 ml. of saturated sodium fluoride solution, the flask being well shaken during each addition. The volume was made up to 250 ml., and the contents after further shaking and standing for an hour were filtered through a fluted filter paper. The filtrate usually contained traces of protein material which gave trouble in the titration of the lactose. Since an increase in the amount of precipitant did not effect an improvement it was necessary to carry out a further precipitation with phosphotungstic and sulphuric acids. The concentration of sulphuric acid was kept sufficiently low to prevent loss of lactose. The filtrate (100 ml.) in a 200 ml. graduated flask was treated with 10 ml. of 2*N* sulphuric acid and 15 ml. of 10 per cent. phosphotungstic acid, made to the mark and filtered. Lactose was determined on a portion of the filtrate. With the quantities mentioned 1 ml. of filtrate corresponded to 0·182 g. of curd, an allowance of 30 ml. being made for the volume occupied by the fat and protein from 50 g. of curd in the first precipitate. Tests of the method on cheese to which known quantities of lactose were added before dissolving in alkali showed that the error in the determination did not exceed 5 per cent. and in many cases was as low as 1 per cent.

*Estimation of lactic acid*

A quantity of emulsion equivalent to 50 g. of curd was treated slowly with 20·5–21·0 ml. of 2*N* sulphuric acid (i.e. slightly in excess of the amount required to neutralize the added alkali) and then with 50 ml. of 10 per cent. copper sulphate solution, the flask being vigorously shaken during each addition. The flask was then heated in a boiling water bath for an hour, the contents were allowed to cool, made up to the 250 ml. mark and then filtered. The

filtrate (150 ml.) was treated with 50 ml. of 10 per cent. milk of lime, bringing the volume to 200 ml., and the mixture after standing 1 or 2 hours was filtered.

As with the lactose estimation in curd, a further precipitation was necessary to remove the last traces of protein and protein hydrolysis products which in this case caused trouble by frothing during the distillation and by reducing large quantities of permanganate. For the reprecipitation, 100 ml. of the filtrate from the lime-copper precipitation were treated with 10 ml. of dilute sulphuric acid (1 : 1) and 5 ml. of 10 per cent. phosphotungstic acid, the volume was made up to 200 ml. and the mixture was filtered. The distillation was carried out on an aliquot containing 10–25 mg. of lactic acid. Allowance was made for the volume of protein and fat in the first precipitate as in the determination of lactose.

With the juices from cheese, or from curd samples taken late in the cheese-making process, it was found necessary to use a second precipitation with phosphotungstic acid as with the curd samples.

The method of determining lactic acid in curd cannot be considered very satisfactory, since variations as high as 10 per cent. between duplicates were frequently encountered, while tests on cheese with added zinc or lithium lactate showed errors as high as 20 per cent. The results obtained have been included in the tables, but it is not felt desirable to rely on them to any great extent in drawing general conclusions.

The accuracy of the determinations of both lactose and lactic acid in whey and juice was quite satisfactory, and good agreement between duplicates was obtained. Tests on salted wheys showed that the presence of large quantities of sodium chloride had no effect on the lactose determination. The error in the lactose estimation was $\pm 1$ per cent. in wheys and only slightly more in juices, while that for the lactic acid estimation was approximately $\pm 5$ per cent.

### *Total solids*

For estimation of total solids in the whey and the curd juice, a volume of 4 ml. was weighed in a flat metal dish and was then dried for 5 hours in the steam oven.

### *Moisture*

For estimation of moisture in the curd, a quantity of 5–10 g. of the sample was heated in a flat metal basin for 4 hours in a steam oven and then for a further 4 hours in an electric oven at 105–110° C.

## Experimental results

A trial experiment on a laboratory scale was made to determine the degree of concordance to be expected from the results of the analytical determinations. Two litres of milk were inoculated with starter culture and coagulated with rennet and thereafter treated according to the normal procedure for cheese making, the various operations being carried out by time rather than

acidity. The weights of milk, starter and rennet solution added were noted and the beaker in which the experiment was carried out was kept covered to reduce evaporation losses. About 1 hour after the main bulk of whey was run off, the curd was drained clear of any further whey, and was then weighed and analysed for moisture, lactose and lactic acid. The whole of the whey was mixed, weighed and analysed. The results permitted a comparison of the total moisture contents of whey and curd with that of the milk and also similar comparisons with regard to lactose and lactic acid.

The values obtained are shown in Table I.

Table I. *Distribution of milk constituents between whey and curd*

| | Total wt. g. | Wt. of moisture g. | Wt. of lactose g. | Wt. of lactic acid g. | Wt. of lactose and lactic acid g. |
|---|---|---|---|---|---|
| Milk and starter | 1884 | 1639 | 86·64 | 0·63 | 87·27 |
| Rennet | 5 | 5 | — | — | — |
| Whey | 1526 | 1419 | 75·63 | 1·30 | 76·93 |
| Curd | 351 | 213 | 8·60 | 0·76 | 9·36 |
| Loss | 12 | 12 | — | — | 0·98 |

The agreement between the moisture determinations is very satisfactory, since the sum of the moisture contents of curd and whey is only 12 g. less than that of the milk, a loss of only 0·75 per cent. of the total amount of moisture, and this loss is exactly accounted for by the loss in total weight of the curd and whey. The difference may thus be due to evaporation and spillage losses. At any rate, the results indicate that discrepancies due to errors in the moisture determinations are negligible.

A loss of 0·98 g. of lactose + lactic acid was found, which amounts to little over 1 per cent. of the total quantity. The smallness of this loss would seem to indicate that the conversion of lactose to lactic acid by starter cultures is almost quantitative in cheese curd as it has been shown to be in milk (3).

In the first experiments on a factory scale, estimations of lactose were made on whey samples obtained throughout the cheese-making process on a number of days. The concentration of lactose in the whey invariably decreased steadily up to the salting stage, after which it showed a very abrupt fall, the differences in the percentage of lactose/water in the wheys immediately before and immediately after salting being about 1·2–1·4, i.e. a 30–35 per cent. fall in concentration. There was a slight rise in the lactose/water concentration in the later wheys after salting. The curve in Fig. 1 is typical of the results obtained. The acceleration in the concentration decrease produced at salting corresponds to the fall in concentration of the mineral constituents already reported (1).

On the basis of the results obtained for lactose, a more comprehensive series of investigations was carried out, analyses being made on samples of whey, curd and juice taken simultaneously at various times during a number of days of cheese making. The results of a typical day's make are presented in graphical form in Figs. 2 and 3.

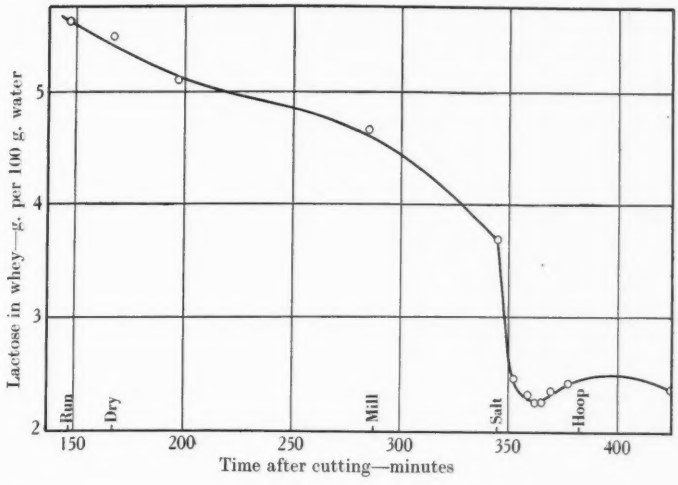


Fig. 1. Variation in concentration of lactose in whey throughout cheese-making process.

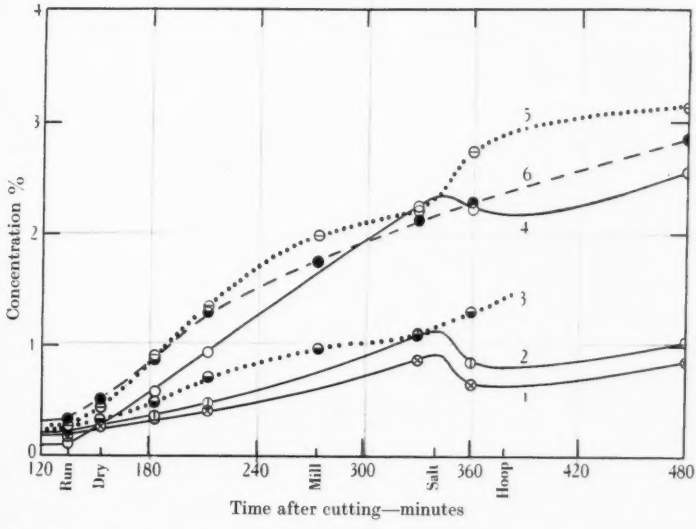


Fig. 2. Variations in acidity and lactic acid in whey, juice and curd throughout cheese-making process: (1) acidity of whey expressed as g. lactic acid per 100 ml. whey; (2) acidity of whey expressed as g. lactic acid per 100 g. water; (3) acidity of juice expressed as g. lactic acid per 100 g. water; (4) lactic acid concentration in whey expressed as g. per 100 g. water; (5) lactic acid concentration in juice expressed as g. per 100 g. water; (6) lactic acid concentration in curd expressed as g. per 100 g. water.

*Acidity*

The curve for acidity/water in whey against time (curve 2, Fig. 2) (acidity is expressed as percentage lactic acid) rises steadily until the salting stage is reached. Thereafter there is a distinct fall, and even in the press whey the acidity does not rise to the value found immediately before salting. The acidity/water concentration of the curd juice (curve 3, Fig. 2) shows no fall after salting but continues to rise steadily. It is thus evident that the fall in acidity in the whey is not due to a fall in acidity in the curd but to some change in the distribution of soluble substances between curd and whey.

It should be noted that the method of expressing the results plotted on the graph as grams per 100 g. of water, automatically corrects for the increase in fat and salt content of the whey or juice. The cheese maker has to base his judgement on the acidity per 100 ml. of whey and the resulting variations may be even more pronounced. For comparison another curve (1, Fig. 2) has been drawn to the same scale showing the acidity expressed as grams lactic acid per 100 ml. of whey. By a coincidence the changes in fat and salt content and specific gravity of the whey almost counterbalance each other so that the two curves are practically parallel. In other instances, however, the variations may be wider. A typical example is given in Fig. 4 which shows acidities of whey obtained from a vat in which cheese was being made from milk of high fat content. Here the abnormally high percentage of fat in the whey near the milling stage caused so much dilution of the other constituents of the whey that a considerable lowering of the acidity resulted.

*Lactic acid*

The concentration of lactic acid (actually lactate ion) in the whey (curve 4, Fig. 2) rises steadily up to the salting stage, the actual values at the later stages of the process being roughly double the corresponding values for acidity calculated as lactic acid. After salting there is a slight fall in lactic acid concentration, much less abrupt than is noticed with the acidity, followed by a slow steady rise.

The lactic acid/water in the curd (curve 6, Fig. 2) shows a steady rise throughout the whole process. The lactic acid/water in the juice (curve 5, Fig. 2) likewise rises throughout the whole process and shows a particularly abrupt rise after salting. In other experiments the rise in lactic acid in the juice was more uniform throughout, but usually there was some acceleration in the rate of rise after salting. Through all the later stages of the process, from shortly after drying onwards, the concentration of lactic acid/water in the juice is somewhat greater than that of lactic acid/water in the curd.

*Lactose*

The curve for lactose/water in the whey (curve 1, Fig. 3) is similar to that already discussed (Fig. 1). The fall in concentration after salting corresponds with the fall in acidity and, as already mentioned, with the fall in concen-

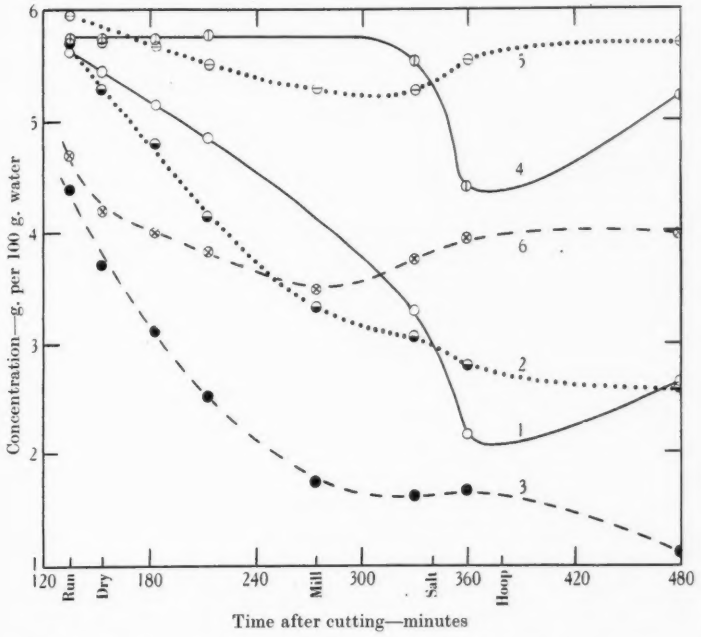


Fig. 3. Variations in lactose and in lactose + lactic acid in whey, juice and curd throughout cheese-making process: (1) lactose in whey; (2) lactose in juice; (3) lactose in curd; (4) lactose + lactic acid in whey; (5) lactose + lactic acid in juice; (6) lactose + lactic acid in curd.

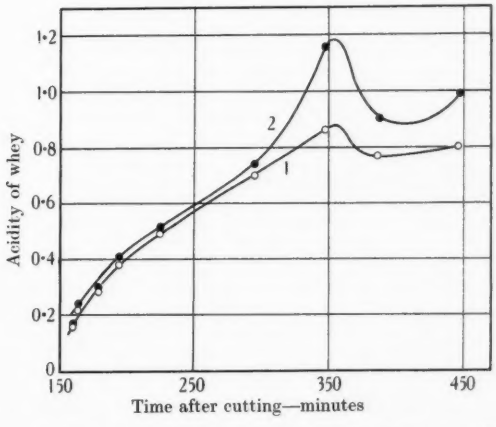


Fig. 4. (1) Acidity expressed as g. lactic acid per 100 ml. whey; (2) acidity expressed as g. lactic acid per 100 g. water in whey.

tration of minerals. It was suggested in an earlier paper(1) that this fall in concentration might be ascribed either to an osmotic diffusion of water from the curd or to a liberation of bound water from the curd by the dehydrating action of the salt.

Except for the samples taken at running and immediately after salting, the lactose/water in the juice is lower than that in the whey. This may be attributed to the fact that a considerable time is required for the whey to separate from the curd and run out of the vat. This time will be greatest where the rate of flow of whey is small. During this period the fermentation of lactose in the curd will have proceeded further, while that in the whey would come almost to a standstill as soon as it left the curd. (It has been shown by Sammis(6) that there is very little acid production in whey when it is separated from the curd.) The same explanation accounts for the fact that the lactic acid content of the juice is higher than that of corresponding wheys.

In the sample taken immediately after salting the lactose in the juice is higher than that in the whey, whereas in the other samples it is lower. This supports the assumption that the fall in concentration after salting is due to an osmotic diffusion of water out of the curd which will thus dilute the whey surrounding the curd particles and cause a concentration of the dissolved substances retained within the curd. A liberation of bound water should dilute both whey and juice equally.

The fact that the lactose/water concentration in the juice is higher than that in the curd is suggestive, particularly as the differences are of the same order throughout all the experiments in this series. A possible explanation is that a portion of the water in the curd is "bound" and is thus not free to act as a solvent for the lactose, which will therefore be concentrated in the free water. Only free water will be removed in the juice, and hence the water in the juice will have a higher lactose concentration than the total water in the curd. From the figures available tentative calculations of the percentage of bound water have been made on the basis of this theory. The method used was as follows:

Let Lactose/water concentration in curd $= L_C$.

,, ,, juice $= L_J$.

Percentage total water in curd $= W$.

,, free ,, $= W_F$.

,, bound ,, $= W_B$.

Then
$$\frac{W_F}{W} = \frac{L_C}{L_J}$$

and
$$\frac{W_B}{W} = \frac{W - W_F}{W} = \frac{L_J - L_C}{L_J},$$

hence
$$W_B = \frac{L_J - L_C}{L_J} W,$$

or, expressed as grams per 100 g. of dry matter in the curd,

$$\text{Bound water} = \frac{L_J - L_C}{L_J} W \frac{100}{100 - W}.$$

The latter method is the most convenient for comparing curds of different moisture contents and has been used for calculation of the results shown in Table II. The results from a second experiment are also included for comparison. The values show considerable fluctuations throughout the course of the cheese-making process, but it is doubtful if these fluctuations are significant. The second experiment indicates a lower general level of bound water than the first. Other experiments gave values lying between these two. There is little evidence of any marked decrease in bound water after salting, and it seems unlikely that any reduction occurring is sufficient to account for the fall in concentration after salting, which must therefore be attributed to osmotic effects.

Table II. *Bound water in curd at various stages of cheese making*

| Process ... | Run | Dry | | | Mill | | Salt | | Hoop | | 1 day old | 2 days old |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time after running (min.) | 0 | 20 | 50 | 80 | 140 | 185 | 215 | 225 | 250 | 450 | — | — |
| % bound water (g. per 100 g. dry matter in curd) | 30 | 30 | 24 | 27 | 31 | 31 | — | 24 | — | 32 | 28 | 32 |

| Process ... | Run | Dry | | Mill | | | Salt | Hoop | | 1 day old | 2 days old |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time after running (min.) | 0 | 15 | 55 | 120 | 135 | 185 | 190 | 240 | 500 | — | — |
| % bound water (g. per 100 g. dry matter in curd) | 20 | 21 | 20 | — | 17 | 17 | — | 19 | 18 | 20 | 21 |

An examination of the figures for concentration of lactose + lactic acid is also of interest. If lactose is converted quantitatively into lactic acid, 1 g. of lactic acid should be formed for every gram of lactose monohydrate fermented, and the weight of lactose + lactic acid in the system should be constant. If both lactose and lactic acid were completely in solution and the amount of bound water in the curd remained constant throughout the process, we should expect to find that the concentration of (lactose + lactic acid)/water would be the same for both whey and juice and would be constant throughout the process. It will be seen from Fig. 3, curve 4, that the values for whey remain constant until the milling stage, after which there is a slight fall with a larger fall after salting, followed by a slight increase to nearly the original value. The value of (lactose + lactic acid)/water in the juice (curve 5, Fig. 3) is practically the same as that of the early wheys and remains fairly constant throughout the process.

The value of lactose + lactic acid/water in the curd (curve 6, Fig. 3) was always lower than that in the juice, presumably for the same reason that the value of lactose/water in the curd was lower than that in the juice.

These results support the contention that the fermentation of lactose to lactic acid in the cheese vat is nearly quantitative.

Analyses were made on the cheese from this and other vats at various times after manufacture. In two normal vats the lactose in the cheese 4 hours after hooping was 0·40 and 0·47 per cent. respectively, and this had fallen to 0·1 per cent. within 4 days and to 0·01–0·02 per cent. within 14 days. In two other vats the amount of lactose incorporated in the cheese was rather higher, being 0·61 and 0·60 per cent. respectively 4 hours after hooping. After 4 days the lactose contents were 0·25 and 0·38 per cent., and at 14 days 0·10 and 0·25 per cent. respectively. The lactic acid rose during the fermentation of the lactose and thereafter remained fairly constant. The longest period for which one cheese was followed was 10 weeks. The results for the first two cheeses are in agreement with those of Van Slyke & Bosworth (4).

*Concentration of lactose in whey at cutting*

It was indicated in the preceding experiment that curd contains a considerable proportion of bound water. If this is true the first wheys obtained after coagulation of milk by rennet should contain a higher lactose/water concentration than is found in the original milk. It was often noticed that this relationship held in the cheese vat. There was a slightly higher concentration of lactose/water in the first wheys than in the milk in spite of the fact that a certain amount of acid had been developed and consequently some of the lactose fermented. The difference, however, was so small as to come almost within the margin of experimental error. A more detailed investigation of this point was made as follows:

A number of samples of milk were taken, either milks of individual cows or mixed milk from various groups of cows. To each 400 ml. sample was added 2·5 ml. of *N* hydrochloric acid and samples were taken for analysis. The milk was then warmed to 32° C. and 0·5 ml. of diluted rennet (1 : 2) added and the beakers transferred to an incubator at 32° C. After 45 min. the curd was cut and the whey poured off through a glass-wool filter. Each 100 ml. of whey was collected and analysed separately. Results from one milk are given in Table III.

Table III. *Relationship of lactose concentrations in milk and whey*

| | Total solids | Density | Fat % | Casein % | Lactose g./100 ml. | Lactose g./100 g. water |
|---|---|---|---|---|---|---|
| Milk | 13·93 | 1·0275 | 4·29 | 2·92 | 5·08 | 5·75 |
| Whey 1 | 7·70 | 1·0250 | — | — | 5·55 | 5·85 |
| Whey 2 | 7·53 | 1·0251 | — | — | 5·56 | 5·86 |
| Whey 3 | 7·31 | 1·0213 | — | — | 5·55 | 5·85 |

The density values are not true densities, but are figures obtained by weighing the amount delivered by a 25 ml. pipette. Since the milk and wheys were measured by a pipette this permitted an accurate conversion of the results to a weight basis. Samples were set up in duplicate for analysis, and two lactose determinations were carried out on each duplicate. The lactose

results were corrected for the volume of fat and casein brought down in the measuring flask in the first precipitation, using the formula

$$\text{Vol. of precipitate} = 1{\cdot}08F + 0{\cdot}74C,$$

where $F$ and $C$ are the weights in grams of fat and casein respectively. For whey the values for $F$ and $C$ were assumed to be 0·2 and 0·9 respectively. Since the correction on the wheys altered the result by only 0·01, any error caused by this assumption is negligible.

With the eleven milks investigated, the concentration of lactose/water in the whey was invariably higher than that in the milk. Although the differences are small they are so consistent that they cannot be ascribed to experimental error. The average of all the determinations expressed as g. lactose/100 g. water was as follows:

Milks, 5·41; Wheys, 5·52.

These figures afford definite support to the conclusion from cheese-making experiments that curd contains "bound" water. Calculation from the average figures given indicated that approximately 2 per cent. of the water in the milks was bound in the curd after coagulation. Taking 6 per cent. as the average content of curd solids in the milk used in the experiments (average fat 3·3 per cent., casein 2·5 per cent.), we arrive at the conclusion that approximately 0·3 g. of water is bound for each gram of curd solids. This compares favourably with the values of 0·15–0·35 found by a different process for curd in cheese making.

*Effect of addition of salt to curd at early stages of the cheese-making process*

A further investigation of the changes in the concentration of whey constituents after salting was made by a study of the effect of the addition of salt to curd taken from a vat at various stages throughout the process. At intervals from the running stage onwards, quantities of 6 or 12 lb. of curd were removed and 2 per cent. by weight of salt was added. The whey exuding from the salted curd was collected and analysed and the results compared with those from analysis of corresponding wheys taken from the unsalted curd in the vat. The experiment was repeated several times. A typical set of results is given in Table IV. In this experiment two vats of the same milk were used, and the proportions of starter added were arranged so as to give a normal rate of acid development in one vat and a fast development in the other.

The results showed that the concentration of lactose/water was always lower in the salted wheys than in the corresponding unsalted wheys. The concentration of lactic acid/water was, however, higher in the salted than in the unsalted wheys. Differences between the concentrations in salted and unslated wheys are shown in Table V.

These differences increase throughout the process. It is to be expected that the addition of salt will have less effect on the curds early in the process, where

the moisture content is high, than later, when the curds contain less moisture. Since the ratio of weight of added salt/weight of curd was kept constant throughout the process, the concentration of salt in the moisture of the curd will be less with the earlier curds than with the later ones. This conclusion is supported by the values found for the salt content of the various wheys.

Table IV. *Effect of salting curd on concentrations of lactose and lactic curd in whey*

| | | Lactose/water | | | | Lactic acid/water | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fast | | Normal | | Fast | | Normal | |
| No. | Sample | Unsalted | Salted | Unsalted | Salted | Unsalted | Salted | Unsalted | Salted |
| 0 | Milk just before renneting | 5·52 | — | 5·49 | — | 0·045 | — | 0·029 | — |
| 1 | Whey 5 min. before running | 5·49 | 4·90 | 5·46 | 4·81 | 0·124 | 0·176 | 0·138 | 0·235 |
| 2 | Whey at drying | 5·15 | 4·49 | 5·15 | 4·36 | 0·390 | 0·447 | 0·350 | 0·371 |
| 3 | Whey ½ hour after drying | 4·57 | 3·13 | 4·65 | 3·06 | 0·544 | 0·862 | 0·777 | 0·863 |
| 4 | Whey 1 hour after drying | 4·38 | 2·63 | 4·46 | 2·40 | — | 1·32 | 1·22 | 1·21 |
| 5 | Whey milling | — | 2·22 | — | 1·97 | — | 1·56 | — | 1·70 |
| 6 | Whey 10 min. after salting | — | 2·13 | — | 1·63 | — | 1·72 | — | 1·75 |
| 7 | Press whey | — | 1·89 | — | 1·41 | — | 1·68 | — | 1·52 |

| | | Lactose + lactic acid/water | | | | NaCl/water in salted whey | | Curd moisture | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fast | | Normal | | | | | |
| No. | Sample | Unsalted | Salted | Unsalted | Salted | Fast | Normal | Fast | Normal |
| 0 | Milk just before renneting | 5·57 | — | 5·52 | — | — | — | — | — |
| 1 | Whey 5 min. before running | 5·61 | 5·08 | 5·60 | 5·04 | 14·8 | 19·2 | 56·74 | 53·27 |
| 2 | Whey at drying | 5·54 | 4·84 | 5·50 | 4·74 | 17·4 | 15·1 | 46·49 | 46·54 |
| 3 | Whey ½ hour after drying | 5·11 | 3·99 | 5·43 | 3·92 | 22·2 | 22·7 | 42·00 | 40·47 |
| 4 | Whey 1 hour after drying | — | 3·95 | 5·68 | 3·61 | 25·2 | 26·8 | 41·46 | 39·95 |
| 5 | Whey milling | — | 3·38 | — | 3·67 | — | — | 40·81 | 39·01 |
| 6 | Whey 10 min. after salting | — | 3·85 | — | 3·38 | — | — | 38·79 | 37·67 |
| 7 | Press whey | — | 3·57 | — | 2·94 | — | — | — | — |

Table V. *Differences*

| | Lactose/water Unsalted minus salted | | Lactic acid/water Salted minus unsalted | |
|---|---|---|---|---|
| No. | Fast vat | Normal vat | Fast vat | Normal vat |
| 1 | 0·59 | 0·65 | 0·052 | 0·097 |
| 2 | 0·66 | 0·79 | 0·057 | 0·021 |
| 3 | 1·44 | 1·59 | 0·318 | 0·086 |
| 4 | 1·75 | 2·06 | — | −0·01 |

In making a comparison of the two vats we find from Table IV that the fall in lactose concentration and rise in lactic acid concentration with salting is almost always greater with the normal vat than with the fast vat. A partial explanation may be found in the fact that throughout the process the moisture content of the curds in the normal vat is fairly consistently less than that of the curd in the fast vat, while the salt concentration in the wheys tends to be higher for the normal vat. It is possible, however, that other factors may also be involved.

*Rate of penetration of salt into curd*

In connexion with the experiments on salting the curd it seemed desirable to determine the rate of penetration of salt into curd. The method used was to apply salt to a flat surface on a slab of curd, and after a definite time to cut a series of slices of known thickness parallel to the salted surface and to determine the salt content of each. The apparatus used had a movable platform to which a curd slab could be clamped and which could be moved through accurately known distances by means of a screw. The portion of the curd slab projecting beyond the platform passed through a rectangular opening in a board faced with glass strips which were used as a guide for a razor. With this apparatus slices of thickness down to 0·02 in. could be cut.

In experiments with curd, suitably sized pieces of curd were cut immediately before milling and left in the cheese vat with the milled curd till near the time for salting. One of the pieces was then put in the slicer and one cut was taken off so as to give a flat surface. The platform was then screwed forward so that the end of the curd slab projected beyond the glass plates and the flat curd surface was dipped in salt crystals. After allowing the desired time for the salt to penetrate, a series of slices was cut from the curd with a razor. The first slice and the outer edges of the other slices were rejected and precautions were taken to prevent salt being carried into the curd from the outside while cutting. The slices were immediately transferred to stoppered weighing bottles and, after weighing, the whole of each slice was transferred to a Kjeldahl flask and the salt content determined by the method of McDowall & Whelan(7).

The results of a typical experiment are shown graphically in Fig. 5. It will be seen that in 15 min. the salt penetrates little more than 0·05 in., and even in $1\frac{1}{4}$ hours there is only a slight rise in salt content 0·15 in. from the surface. It is evident, therefore, that but little salt will have reached the centre of each strip of curd by the time the curd is normally put in the press.

A further experiment was carried out to determine the uniformity of salt distribution in cheese after pressing. A number of cubes of curd about 3 in. side were cut before the rest of the curd was milled and were left in the vat with the milled curd, salted with it and pressed surrounded by curd strips of the normal size. On the following day the cheeses were taken from the press, broken up, and the large slabs of curd removed. A flat surface was formed on each slab by pressing it with weights for 1 or 2 hours on a glass plate. The other edges of the slabs were then trimmed off so as to minimize the effect of diffusion from surfaces other than the reference surface. A series of slices were then cut parallel to the flat surface and the salt content of each determined. Owing to the thickness of these slices their weight was too great for analysis by the McDowall & Whelan method. Each slice was therefore dissolved in sodium hydroxide and the chloride determined in an aliquot by the van der Burg method(7).

Experimental results are shown in Fig. 6. Slices were cut in two planes, (*a*) parallel to the grain of the curd, and (*b*) at right angles to the grain. It will be seen that the salt has penetrated to a greater depth along the grain than at right angles to it. This is quite to be expected, since the curd is more

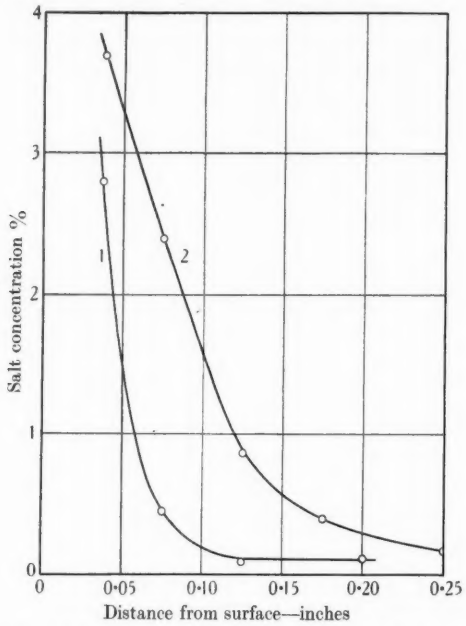


Fig. 5. Penetration of salt into curd: (1) 15 minutes' penetration; (2) 75 minutes' penetration.

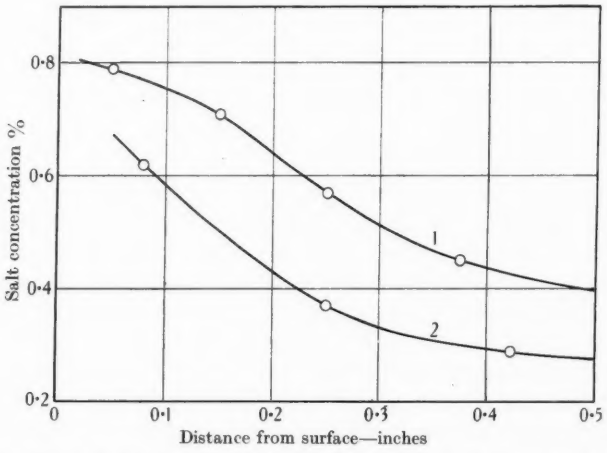


Fig. 6. Penetration of salt into cheese one day after hooping; (1) Curd cut at right angles to grain; (2) curd cut parallel to grain.

porous along the grain. It will also be seen that even ½ in. from the surface there is a considerable rise in salt content. Since the milled strips of curd have a thickness of the order of only 0·3 in. at hooping, it is evident that, within less than 1 day after pressing, the salt will have penetrated into the centre of the curd particles.

These results are compatible with the findings of McDowall & Whelan(8) on the rate of diffusion of salt in Cheddar cheese. No direct comparison, however, is possible, since in the earlier investigation the times allowed for diffusion were much greater, the shortest being 7 days. Both investigations, however, have shown that the rate of diffusion of salt into cheese curd is very slow.

*Attempted estimation of bound water in curd*

Experiments described earlier in this paper have indicated the presence of bound water in cheese curd, and it was thought desirable to seek more direct evidence on the subject. Of the various methods which have been used for determining bound water in biological material few appear suitable for use with cheese curd. Three general principles have been used:

(*a*) The measurement of non-freezing water by determination, either by dilatometric or calorimetric means, of the ice formed on freezing the sample.

(*b*) Determination by direct analysis of the concentration of a reference substance in the free and in the total water in the material investigated. If the substance is insoluble in the bound water its concentration in the free water will be greater than that in the total water, and from the two values the percentage of bound water may be calculated.

(*c*) Calculation of the concentration of a reference substance in the free water in the material by measurements of the freezing-point depression or vapour-pressure lowering produced by the substance.

Method (*c*) involves difficulties in getting the reference substance uniformly diffused through the curd sample, and the bacterial action which would occur during this process, and the subsequent measurement would be sufficient entirely to invalidate the results unless the whole determination could be carried out at a temperature sufficiently low to stop bacterial action. Method (*a*) would avoid this difficulty provided the cooling were commenced immediately after the curd was sampled. The use of low temperatures with either method may, however, be objected to on the ground that when measurements are made at a temperature of 30–50° C. below that used in cheese making, there must be some doubt as to whether the results are applicable at the higher temperature.

It was decided to use method (*b*) and to add the reference substance to the milk at the beginning of the cheese-making process and to estimate its concentration in the wheys.

It was necessary to select some substance which would not be toxic to the starter bacteria in the cheese vat and which would not be acted on by them and which could be accurately estimated in small concentrations in whey.

It was also important that the reference substance should not react with any of the substances found in the cheese vat to form insoluble compounds, that it should not be soluble in fat and that it should not be adsorbed by protein.

Sodium sulphate was chosen for use in the first experiments. The sulphate ion was estimated gravimetrically as barium sulphate after precipitation of the protein with phosphomolybdic and hydrochloric acids. A solution containing about 15 per cent. $Na_2SO_4$ was added to the milk in the vat at the commencement of cheese making. The quantity used was such as to give a concentration of about 0·1 per cent. in the water in the milk. This concentration was sufficiently low to avoid precipitation as calcium sulphate at the temperature of the cheese vat. A control vat was run on the same milk, and to this was added the same quantity of water as had been added to the other vat. Sodium chloride, free from sulphate, was used in salting the curd from both vats so that no additional sulphate should be introduced from this source. A series of analytical results for one experiment is shown in Table VI.

Table VI. *Concentration of sulphate in wheys*

Vat 1: 800 lb. milk + 4000 ml. water.
Vat 2: 800 lb. milk + 4000 ml. $Na_2SO_4$ solution.

| Sample No. | Description | Total solids | | Density | | Sulphate/water | | |
|---|---|---|---|---|---|---|---|---|
| | | Vat 1 | Vat 2 | Vat 1 | Vat 2 | Vat 1 | Vat 2 | Diff. |
| 0 | Milk | 12·95 | 12·98 | 1·0254 | 1·0272 | 0·0049 | 0·1123 | 0·1074 |
| 1 | Whey just after cutting | 7·33 | 7·53 | 1·0225 | 1·0248 | 0·0056 | 0·1133 | 0·1077 |
| 2 | Whey at running | 7·13 | 7·33 | 1·0211 | 1·0227 | 0·0052 | 0·1150 | 0·1098 |
| 3 | Whey at drying | 6·94 | 7·16 | 1·0225 | 1·0246 | 0·0078 | 0·1152 | 0·0174 |
| 4 | Whey ½ hour after drying | 7·02 | 7·24 | 1·0247 | 1·0255 | 0·0069 | 0·1133 | 0·1064 |
| 5 | Whey 1 hour after drying | 7·30 | 7·34 | 1·0260 | 1·0272 | 0·0057 | 0·1091 | 0·1034 |
| 6 | Whey just before salting | 8·25 | — | 1·0301 | — | — | — | — |
| 7 | Whey just after salting | 23·91 | 23·83 | 1·1504 | 1·1469 | 0·0062 | 0·1513 | 0·1451 |
| 8 | Whey after salting | 22·74 | 22·58 | 1·1211 | 1·1306 | 0·0101 | 0·1675 | 0·1564 |
| 9 | Whey after salting | 19·64 | 19·10 | 1·0585 | 1·0573 | 0·0113 | 0·1613 | 0·1500 |

The concentration of sulphate/water in the first wheys approximated closely to that in the milk and remained practically constant up to salting. After salting, instead of the fall in concentration which had been noticed with other soluble constituents of whey, there was a considerable rise in the concentration of sulphate, amounting to approximately 50 per cent. Tests on the analysis of wheys after salting and on wheys with added sodium chloride indicated that no appreciable error in the sulphate estimation was introduced by high concentrations of sodium chloride. A possible explanation of the rise in concentration of sulphate after salting is that a Donnan equilibrium controls the distribution of ions, and that while the concentration of cations such as calcium falls that of anions rises. This theory will be discussed more fully at the end of this paper (see also (11)).

It became evident from the above results that electrolytes were unsuitable for use as reference substances for determining changes in the amount of bound water in curd. Considerable difficulties arose in the selection of a suitable non-electrolyte for the purpose, and urea appeared to be the only

substance available which complied with the conditions. This was added to milk as already described, and was determined in whey by the method of Conway (9). In some experiments the urea of the milk (0·02–0·04 per cent.) was used without further addition. A fall in concentration of urea in the whey was, however, invariably found throughout the cheese-making process. Further investigations made it clear that the urea was converted to ammonia by some enzyme either originally present in the milk or produced by the starter bacteria. Rennet was found to have no action on urea.

Attempts to find other suitable reference substances have so far been unsuccessful and the investigation has had to be postponed until such time as other methods can be tested out.

## General discussion

### (*a*) *Bound water in curd*

The experiments involving estimation of lactose in curd and curd juice have given some evidence of the existence of bound water in cheese curd. Further support for this suggestion is furnished by the results of experiments on the concentrations of lactose in milk and in wheys obtained shortly after cutting the curd. These experiments together indicate that, of the water in the curd, an amount of the order of 0·2–0·3 g. per g. of dry matter in the curd is bound. Attempts to obtain more direct evidence have been unsuccessful up to the present but are to be continued later. The presence of bound water in cheese curd should be of importance, not only in determining the rate of separation of moisture and dissolved substances in the whey, but also in affecting the rate and extent of drying out of cheese.

### (*b*) *Membrane equilibria*

There is definite evidence of the existence of a Donnan equilibrium which controls the partition of ions between curd and whey and which also involves an osmotic effect which determines the concentration of non-electrolytes after salting. It has been shown that after salting there is a pronounced fall in the concentration of calcium, a much less pronounced fall in phosphate and lactate, and a rise in sulphate in the wheys. With curd salted at an earlier stage than normal, the whey contained a higher concentration of lactate/water than did the whey from another portion of the same curd which had not been salted. Summing up these results we may say that after salting there is an osmotic diffusion of water from the curd tending to dilute all the constituents of the whey, while there is also a membrane effect which tends to increase the concentration of anions and decrease that of cations.

If we imagine a curd particle as being surrounded by a membrane permeable to all ions but protein ions, we obtain the conditions necessary for a Donnan equilibrium. It may be shown theoretically for such a case that the addition of an electrolyte such as sodium chloride will produce a rise in

concentration of the anions and a fall in concentration of cations in the outer liquid. This is what has actually been found to be the case in the cheese vat at salting.

The outward diffusion of water may be another result of this equilibrium or may be a simple osmotic diffusion due to the slow rate of diffusion of salt into the curd. The concentrations of sodium chloride inside and outside the particle may be brought into equilibrium either by an outward diffusion of water or by an inward diffusion of sodium chloride. Until equilibrium is established, which may require some hours, both forms of diffusion will probably take place. Much of the water which diffuses out will escape as whey and will contain the various whey constituents in a more dilute form than prior to salting.

The Donnan equilibrium will have an important effect in determining the relation of the lactic acid in the curd to the whey acidity at all stages of the process. Any factors affecting this equilibrium will thus alter the significance of the whey acidities as a means of determining the extent of lactose fermentation in the curd. Among the factors controlling the equilibrium is the ionization of the curd protein. This will be affected by *p*H and also probably by other factors such as heat and rennet action, which alter the nature of the protein. It seems possible, therefore, that in this equilibrium lies the key to the anomalous acidity readings mentioned in the introduction to this paper. Variations in the percentage of bound water in the curd as a result of similar influences would also have an important effect, but there is not yet sufficient evidence on the subject to bring this forward as a likely suggestion.

## Summary

1. Determinations have been made of lactose and lactic acid in whey, curd and curd juice throughout the cheese-making process.

2. The effect of adding salt to curd at various stages of the process on the concentration of lactose and lactic acid in the whey has been investigated.

3. The rate of penetration of salt into curd has been determined, and it has been shown that by the time the cheese is normally hooped but little salt has reached the centre of each curd strip. Within 12 hours after hooping the distribution of salt is substantially uniform.

4. It has been shown that the conversion of lactose to lactic acid by starter bacteria in the cheese vat is almost quantitative.

5. Evidence has been brought forward indicating the presence of bound water in cheese curd.

6. It has been shown that a Donnan equilibrium controls the partition of electrolytes between curd and whey.

Acknowledgements

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# 136. ACID PRODUCTION AND PROTEIN DEGRADATION OF SOME ACID-PROTEOLYTIC COCCI

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Various types of proteolytic cocci, including several acid-proteolytic strains, have been isolated from foremilk and rennet extract (1) and subsequently from ripened Cheddar cheese. A study of the proteolytic activities of these cultures in milk forms the subject of this investigation.

It was decided to determine, (*a*) the degradation of casein to water-soluble nitrogen compounds by the above cultures, and (*b*) the increase of amino nitrogen. At the same time, acidity and *p*H determinations were made at regular intervals to aid in the classification of the cultures.

The cultures investigated are described in Table I, their origin and probable nomenclature being included. Prior to the investigation a number of the cultures had been propagated on artificial media for periods of 6–12 months.

## Experimental

### *Preparation of the milk*

The milk used in the investigation was obtained from the same bulk quantity and the whole treated identically. Freshly separated milk contained in 500 ml. flasks and sterilized by steaming at 100° C. for 30 min. on three consecutive days was used. Each flask contained 300 ml. of milk. In addition, a series of large saline tubes containing 15 ml. milk were prepared to be used for inoculation purposes. The above quantities were carefully adhered to so that evaporation losses would be constant. The milk was proved free from viable bacteria by incubation and plating.

### *Inoculation of the milk*

In order that the milk should be inoculated with young vigorous cells, 24-hour-old growths were used. A heavy saline suspension was prepared from the 24-hour nutrient agar streak and 1 ml. inoculated into a tube containing 15 ml. milk. This tube was incubated at 30° C. for 24 hours, stained preparations being made to determine the extent of growth. If sufficient cells were present, and this was usually the case, 10 ml. of the milk culture were abstracted with a sterile 10 ml. pipette and inoculated into a flask containing 300 ml. milk. This bulk milk was then incubated at 30° C. for a further 24 hours. Preliminary experiments showed this period to be quite suitable for ensuring vigorous growth in the milk without clotting. In the case of

cultures of *Streptococcus liquefaciens*, the 24-hour period had to be shortened to one of 12 hours, on account of the curdling so rapidly produced.

After 24 hours, slides were prepared from the 300 ml. inoculated milk, and if growth was satisfactory the milk was distributed in 50 ml. quantities into four large sterile boiling tubes and incubated at 30° C. After 2 days the tubes were examined separately for purity.

The tubes were incubated at 30° C. and the contents examined at weekly intervals for 4 weeks. Each of the listed cultures in Table I was treated exactly as above, a series of four milk tubes being prepared for each. A control set of tubes containing 50 ml. of sterile milk was incubated with the culture tubes and analysed at similar intervals. An attempt to combine optimal conditions for saccharolysis and proteolysis was made by incubation at 30° C., at which the cultures had previously been shown to grow vigorously.

*Treatment of milk cultures for chemical examination*

At each examination period of 1, 2, 3 and 4 weeks, the milk cultures from the boiling tubes were transferred quantitatively to a clean mortar, using as little distilled water as practicable for washing out the residues. The mass was then ground steadily until it was homogeneous. In some cases it was time consuming, but a homogeneous mixture was always secured.

For the chemical investigation 40 ml. of the ground suspension were pipetted into a 100 ml. flask and the volume made up to the mark with distilled water. An additional 5 ml. of the same suspension were also pipetted into a sterile test-tube and reserved for *p*H determination. Investigations were carried out immediately on the 100 ml. milk suspension to avoid inaccuracies caused by contaminants.

*Chemical investigation*

The following fractions were determined by the usual methods:

| | |
|---|---|
| Total nitrogen ... ... ... | T.N. |
| Protein nitrogen ... ... ... | P.N. |
| Non-protein nitrogen ... ... | N.P.N. |
| Amino nitrogen ... ... ... | T.N. and N.P.N. (Sørensen) |

*p*H *determinations*

*p*H determinations were made by the quinhydrone method.

All figures for the various nitrogen fractions have been expressed as milligrams of nitrogen per 100 ml. of the original culture. In Table II it will be seen that evaporation was considerable and caused a steady increase in the amount of total nitrogen per sample. In certain cases cultures show a decrease in the amount of total nitrogen compared with the previous determination, because additional distilled water had to be used to wash out quantitatively the residues from the boiling tubes. Acidity figures were obtained from the first reading of the total amino-nitrogen titration, and are expressed as percentage lactic acid.

Table I. *Description of the proteolytic cocci investigated*

| Culture | Source | Probable nomenclature | Carbohydrate fermentations | | | | | | | | Nitr. red. | Gel. liq. | Action in lit. milk | | | Pigment | Haemolysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Dext. | Sucr. | Lact. | Malt. | Mann. | Inul. | Salic. | Raff. | | | Acid. | Coag. | Pep. | | |
| 1 | Udder | *Str. liquefaciens* | + | + | + | + | + | − | + | − | − | + | + | + | + | — | − |
| 2 | Udder | *Staph. aureus* | + | + | + | + | + | − | − | − | + | + | + | + | + | Orange | + |
| 3 | Udder | *M. caseolyticus* | + | + | + | + | + | − | − | − | + | + | + | + | + | Lemon-yellow | − |
| 4 | Udder | *M. freudenreichii* | + | + | + | + | − | − | − | − | − | + | + | + | + | Lemon-yellow | − |
| 5 | Rennet | *Staph. aureus* | + | + | + | + | − | − | − | − | − | + | + | + | + | Orange | + |
| 6 | Rennet | *M. caseolyticus* | + | + | + | + | − | − | − | − | + | + | + | + | + | Pale yellow | − |
| 7 | Rennet | *Str. liquefaciens* | + | + | + | + | + | − | + | + | − | + | + | + | + | — | − |
| 8 | Rennet | *Staphylococcus* sp. | + | − | − | − | − | − | − | − | − | + | + | + | + | Yellow | + |
| 9 | Rennet | *M. freudenreichii* | + | + | − | − | + | − | + | − | − | + | − | + | + | Chalk white | − |
| 10 | Cheese | Unnamed | + | − | + | + | − | − | − | − | + | + | + | + | + | Yellow | − |
| 11 | Cheese | *Staph. aureus* | + | + | + | + | + | − | − | − | + | + | + | + | + | Orange | + |
| 12 | Cheese | *Staph. aureus* | + | + | + | + | + | − | − | − | + | + | + | + | + | Orange | + |
| 13 | Ripened Cheddar | Unnamed | + | + | + | + | + | − | − | − | + | + | + | + | + | Orange | Slight |
| 14 | Ripened Cheddar | Unnamed | + | + | − | + | + | − | − | − | + | + | − | + | + | — | Slight |

Table II. *Nitrogen distribution in cultures*

| Culture | Total | As % of total nitrogen: Protein N | N.P.N. | T.N. Sørensen | N.P.N. Sørensen | *p*H | Acidity as % lactic acid |
|---|---|---|---|---|---|---|---|
| Control | | | | | | | |
| 1st week | 549·50 | 92·81 | 7·19 | 6·12 | 3·82 | 6·43 | 0·2013 |
| 2nd week | 575·75 | 92·97 | 7·03 | 7·14 | 4·13 | 6·50 | 0·1725 |
| 3rd week | 609·50 | 92·74 | 7·26 | 7·01 | 3·90 | 6·47 | 0·1825 |
| 4th week | 630·00 | 92·62 | 7·38 | 6·83 | 3·77 | 6·50 | 0·1750 |
| Culture No. 1 | | | | | | | |
| 1st week | 549·50 | 24·57 | 75·43 | 9·05 | 8·92 | 4·60 | 1·0413 |
| 2nd week | 580·50 | 23·17 | 76·83 | 8·23 | 7·80 | 4·55 | 1·1425 |
| 3rd week | 601·75 | 22·85 | 77·15 | 11·55 | 10·22 | 4·69 | 1·0413 |
| 4th week | 589·00 | 21·05 | 78·95 | 11·27 | 10·74 | 4·76 | 1·0450 |
| Culture No. 2 | | | | | | | |
| 1st week | 559·00 | 72·76 | 27·24 | 8·32 | 6·08 | 5·00 | 0·6700 |
| 2nd week | 535·25 | 66·56 | 33·44 | 10·39 | 7·38 | 4·97 | 0·6400 |
| 3rd week | 601·75 | 68·22 | 31·78 | 10·49 | 8·02 | 5·01 | 0·7360 |
| 4th week | 619·25 | 63·91 | 36·09 | 10·82 | 7·91 | 5·11 | 0·7300 |
| Culture No. 3 | | | | | | | |
| 1st week | 546·25 | 81·83 | 18·17 | 6·43 | 3·75 | 5·65 | 0·3663 |
| 2nd week | 570·25 | 81·02 | 18·98 | 8·72 | 6·05 | 5·70 | 0·4213 |
| 3rd week | 601·75 | 80·22 | 19·78 | 8·73 | 6·27 | 5·53 | 0·4613 |
| 4th week | 590·50 | 74·64 | 25·36 | 8·83 | 6·56 | 5·63 | 0·4113 |
| Culture No. 4 | | | | | | | |
| 1st week | 525·75 | 84·04 | 15·96 | 6·85 | 3·19 | 5·40 | 0·4513 |
| 2nd week | 513·00 | 79·09 | 20·91 | 8·26 | 5·41 | 5·43 | 0·4263 |
| 3rd week | 573·25 | 79·20 | 20·80 | 9·09 | 6·24 | 5·39 | 0·4850 |
| 4th week | 626·75 | 74·30 | 25·70 | 8·94 | 5·90 | 5·53 | 0·4875 |
| Culture No. 5 | | | | | | | |
| 1st week | 546·25 | 87·19 | 12·81 | 6·32 | 4·94 | 5·65 | 0·3675 |
| 2nd week | 576·50 | 82·13 | 17·87 | 5·90 | 2·99 | 5·20 | 0·5913 |
| 3rd week | 518·75 | 79·71 | 20·29 | 7·86 | 4·87 | 5·08 | 0·5013 |
| 4th week | 583·50 | 73·23 | 26·78 | 8·12 | 5·57 | 5·29 | 0·5475 |
| Culture No. 6 | | | | | | | |
| 1st week | 550·25 | 68·88 | 31·12 | 7·18 | 6·32 | 5·33 | 0·5688 |
| 2nd week | 593·50 | 58·43 | 41·57 | 6·47 | 6·53 | 5·20 | 0·6825 |
| 3rd week | 593·00 | 55·31 | 44·69 | 9·59 | 7·38 | 5·25 | 0·5888 |
| 4th week | 581·00 | 50·95 | 49·05 | 9·92 | 8·31 | 5·36 | 0·5988 |
| Culture No. 7 | | | | | | | |
| 1st week | 559·75 | 38·46 | 61·54 | 8·75 | 8·13 | 5·10 | 0·7350 |
| 2nd week | 593·50 | 31·68 | 68·32 | 7·75 | 7·71 | 4·90 | 0·8838 |
| 3rd week | 592·25 | 30·98 | 69·02 | 11·21 | 10·38 | 4·97 | 0·8475 |
| 4th week | 572·25 | 30·63 | 69·37 | 10·88 | 9·92 | 4·98 | 0·8125 |
| Culture No. 8 | | | | | | | |
| 1st week | 544·50 | 72·97 | 27·03 | 8·77 | 5·65 | 6·30 | 0·3025 |
| 2nd week | 581·00 | 57·23 | 42·77 | 10·61 | 8·43 | 6·15 | 0·4550 |
| 3rd week | 599·25 | 51·40 | 48·60 | 18·32 | 15·02 | 6·03 | 0·3838 |
| 4th week | 574·75 | 47·98 | 52·02 | 20·12 | 17·18 | 6·10 | 0·4000 |
| Culture No. 9 | | | | | | | |
| 1st week | 543·25 | 89·09 | 10·91 | 6·26 | 4·65 | 6·33 | 0·2225 |
| 2nd week | 563·25 | 81·85 | 18·15 | 6·39 | 4·66 | 6·53 | 0·2388 |
| 3rd week | 576·50 | 63·58 | 36·42 | 8·78 | 6·33 | 6·64 | 0·2013 |
| 4th week | 605·50 | 58·96 | 41·04 | 9·99 | 8·09 | 6·85 | 0·1975 |
| Culture No. 10 | | | | | | | |
| 1st week | 535·25 | 87·30 | 12·70 | 6·94 | 4·72 | 5·60 | 0·4300 |
| 2nd week | 500·50 | 77·27 | 22·73 | 8·47 | 6·19 | 5·32 | 0·4138 |
| 3rd week | 528·25 | 80·69 | 19·31 | 8·92 | 6·77 | 5·36 | 0·4850 |
| 4th week | 564·75 | 77·11 | 22·89 | 9·23 | 6·15 | 5·28 | 0·5375 |
| Culture No. 11 | | | | | | | |
| 1st week | 540·25 | 71·77 | 28·23 | 7·75 | 6·43 | 5·07 | 0·6588 |
| 2nd week | 568·50 | 64·99 | 35·01 | 10·55 | 7·70 | 4·95 | 0·6850 |
| 3rd week | 593·00 | 65·18 | 34·82 | 11·19 | 8·43 | 4·98 | 0·7100 |
| 4th week | 594·00 | 60·40 | 39·60 | 11·87 | 8·50 | 5·15 | 0·7163 |

Table II (*continued*)

| Culture | Total | As % of total nitrogen | | | | *p*H | Acidity as % lactic acid |
|---|---|---|---|---|---|---|---|
| | | Protein N | N.P.N. | T.N. Sørensen | N.P.N. Sørensen | | |
| Culture No. 12 | | | | | | | |
| 1st week | 553·25 | 75·24 | 24·76 | 7·28 | 6·15 | 5·07 | 0·6575 |
| 2nd week | 549·50 | 70·70 | 29·30 | 10·28 | 7·05 | 4·97 | 0·6450 |
| 3rd week | 595·25 | 68·63 | 31·37 | 10·61 | 7·35 | 5·05 | 0·7200 |
| 4th week | 590·75 | 67·29 | 32·71 | 10·96 | 7·99 | 4·98 | 0·7288 |
| Culture No. 13 | | | | | | | |
| 1st week | 553·75 | 82·53 | 17·47 | 6·64 | 3·59 | 6·50 | 0·2113 |
| 2nd week | 584·25 | 72·19 | 27·81 | 6·76 | 5·05 | 6·58 | 0·2538 |
| 3rd week | 611·00 | 68·62 | 31·38 | 9·04 | 7·16 | 6·84 | 0·1975 |
| 4th week | 584·25 | 65·94 | 34·06 | 10·40 | 8·26 | 6·77 | 0·1813 |
| Culture No. 14 | | | | | | | |
| 1st week | 555·00 | 82·48 | 17·52 | 6·56 | 3·29 | 6·50 | 0·2213 |
| 2nd week | 574·00 | 71·69 | 28·31 | 7·10 | 5·40 | 6·45 | 0·2525 |
| 3rd week | 609·50 | 59·11 | 40·89 | 9·09 | 8·94 | 6·78 | 0·2225 |
| 4th week | 611·75 | 56·31 | 43·69 | 11·81 | 10·26 | 6·73 | 0·2225 |

## Discussion

The results obtained showed the methods to be satisfactory for determining the protein degradation of milk by micro-organisms. Duplicates were found to be in close agreement, showing that the grinding and agitation procedure had largely eliminated the sampling error. The only discrepancy encountered was that the extent of the protein breakdown appeared to vary slightly in individual culture tubes, a lower figure being recorded after 3 weeks' incubation than for the same culture at 2 weeks. This discrepancy was also noticed in the case of the amino-nitrogen fraction, and in this particular case it may have been the result of the utilization by bacteria of a portion of the amino nitrogen already formed. This discrepancy was quite small and not of sufficient magnitude to interfere with the interpretation of the results.

The two cultures of *Str. liquefaciens* investigated were by far the most active proteolytic types. Both cultures brought about the major portion of the protein breakdown during the first week's incubation, yielding only a slight increase on further holding. The final breakdown figures for each type, after deducting the control figures, were:

*Str. liquefaciens*, from the udder, 70·5 per cent.

*Str. liquefaciens*, from rennet, 62·0 per cent.

Both cultures gave a slight but steady increase in the amount of amino nitrogen present, the major portion being in the form of non-protein amino nitrogen. From the results obtained it appears that *Str. liquefaciens* restricts its proteolytic activities to the formation of water-soluble decomposition products from the casein, the amino-nitrogen content of the milk being but little increased. Acid production in both cases was strong, the cultures being readily recognized as acid-proteolytic.

Of the staphylococci investigated, two types were recognized according to

their effect on the amino-nitrogen fraction of the milk. The orange type produced but little increase in the amino-nitrogen content, approximately 1–5 per cent. over that of the control milk, but the yellow haemolytic type, culture No. 8, produced the comparatively large increase of 13 per cent. Furthermore, culture No. 8 was proteolytic to the extent of 45 per cent., whereas the orange types degraded the casein approximately 20–32 per cent. The orange staphylococci appeared to restrict their activities to the decomposition of protein to intermediate water-soluble compounds, and, excepting the yellow strain investigated, the effect of the haemolytic cocci on the amino-acid content of the milk appeared to be very slight. The orange strains produced more acid in milk than the yellow types, but all could be described as acid-proteolytic.

The two strains of *Micrococcus caseolyticus* investigated showed wide differences in their ability to attack casein. The culture obtained from the foremilk (culture No. 3) had been previously shown to have decreased in proteolytic activity after artificial culture, and this was recognized during the chemical investigation where only 18 per cent. of the casein was decomposed. The strain from rennet was more active and broke down protein to the extent of 42 per cent. Both types failed to cause any marked increase in the amino-nitrogen content of the milk, and the cultures restricted their activities to the formation of intermediate water-soluble decomposition products. Acid production in both strains was sufficient to classify them as acid-proteolytic.

The two cultures of *M. freudenreichii* investigated made but little difference to the amino-acid content of the milk. Culture No. 4 from the udder decomposed the protein present to the extent of 18·7 per cent., culture No. 9 to 34 per cent. The latter culture, isolated from rennet, was decidedly ropy and slimy in milk, but failed to produce acid.

Cultures Nos. 13 and 14 failed to produce acid. Both were proteolytic to the extent of 27 and 36 per cent. respectively, but were only capable of increasing the amino-acid content of the milk 3·5 and 4·5 per cent.

Culture No. 10 decomposed the milk protein to the extent of 16 per cent., but possessed no power to increase the amino-nitrogen fraction. Acid production was poor and the culture only weakly acid-proteolytic.

The organisms described have been shown in many cases to produce significant degradations in the protein of milk at *p*H values such as are found in ripening cheese. They may therefore if present in sufficient numbers contribute to the general protein degradation, but it is likely that their presence is undesirable because of the possible effect upon flavour.

The author wishes to thank Dr A. T. R. Mattick for his interest in this work and for many helpful suggestions.

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637.133.3:576.8

# 137. STUDIES IN THE BACTERIOLOGY OF LOW-TEMPERATURE PASTEURIZATION

## PART II
## THE HEAT RESISTANCE OF A THERMODURIC *STREPTOCOCCUS* GROWN AT DIFFERENT TEMPERATURES

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(With 1 Figure)

Since the publication on physiological youth in bacteria by Sherman & Albus(1), many workers have demonstrated that the resistance of micro-organisms to environmental changes is greatly influenced by the age or stage of growth of the organism under consideration.

Sherman & Albus(2) reported a decreased resistance of *Bact. coli* to 5 per cent. NaCl just before and during the early stages of active growth. From their findings these authors concluded that the mature cells from old cultures assume the characteristics of young cells before reproduction begins—the physiologically young cells being very much less resistant to an adverse environment than the more mature cells. Robertson(3), working with a heat-resisting *Streptococcus*, inoculated a 7-hour culture at 25° C. into sterile milk at the same temperature. The culture was kept at 25° C., and portions were heated at 62·8° C. for 30 min. at regular intervals. Robertson found that the young rapidly growing cells were more susceptible than the older cells to the killing action of high temperatures, and from this he concluded that the most efficient pasteurization of milk can be obtained if the heat is applied before the micro-organisms have passed the accelerative growth stage. Stark & Stark(4), working with cultures of *Str. faecalis* at 37° C., found that a 24-hour culture was more resistant than a 4-hour culture. Using a ropy milk organism cultivated at room temperature the same workers found that a 54-hour culture was more resistant than a 24-hour culture which itself was more resistant than a 6-hour culture. In every case the young cells were markedly less resistant to heat than were the mature cells of the same organism. Hammer & Hussong(5) found that a 7-hour culture of a ropy milk organism was killed, while a 79-hour culture resisted a temperature of 62·8° C. for 10 min. Fabian & Coulter(6) report that young cultures of *Bact. coli* (6 and 12 hours) were more susceptible to heat than old cultures (24 hour) when heated in an ice-cream mix at 61·6° C. for 30 min.

The effect of growth temperatures and temperature of incubation on heat resistance has been studied by several workers. Magoon(7), working with

spores of *Bact. mycoides*, found that storage at a temperature somewhat below the optimum and well above the minimum for vegetative growth seemed to be most favourable for increase in spore resistance. The increase in resistance of spores was found to take place most rapidly under conditions suited to positive but rather sluggish vital activities normal to the spore form. Curran (8) also studied bacterial spores and found that the thermal resistance was increased with the temperature at which they were held up to the optimum temperature of the organism. Sherman *et al.* (9) demonstrated that milk stored for 24 hours at 15° C. gives a greater percentage reduction as the result of pasteurization (62·8° C. for 30 min.) than the same milk stored at 4·4° C. But it should be noted that in the tables reproduced by the authors there is little difference in the actual numbers of heat-resisting organisms, the increased percentage reduction being accounted for by an enormously increased bacterial content of the unheated milk after storage at 15° C. Stark (10) reports that ropy milk outbreaks have been eliminated by permitting raw milk to remain in the receiving tank at a temperature of 26–32° C. long enough for the mature cells to become physiologically young before the milk was pasteurized. Sherman & Cameron (11, 12), investigating the environmental change taking place within the normal growth range of *Bact. coli*, suggest that "slow growth should lead to a more perfect adjustment of an organism to its environment and, therefore, to greater viability". The same authors (13) have recently published a note reporting increased viability when the growth rate of organisms is reduced by growth at low temperatures, growth in a dilute medium, or growth in a medium of increased osmotic pressure.

Walker *et al.* (14) report an increase in cell size and increased metabolic activity at the close of the lag and at the beginning of the log phase of growth. The authors connect this increased activity with susceptibility to heat described by Sherman & Albus (1).

From this short review of some of the more recent publications it is evident that with the initiation of growth in a bacterial culture there is a corresponding decrease in resistance to severe environmental changes, the resistance of the young cell being least when the rate of multiplication is greatest.

## Present investigation

In our previous paper (15) we dealt with the presence and origin of thermoduric organisms in raw milk and their significance in the process of pasteurization. In view of the importance of at least some of the species, we decided to make a more minute study of those isolated, particularly of their physiology and biochemistry. This paper represents the first contribution on these lines and deals with the effect of growth temperature on heat resistance. In view of the unexpected results it has been decided to put some of the work on record at an early stage.

The organism used in the experiments to be described was isolated after

laboratory pasteurization of milk from a farm sending its supplies direct to a pasteurizing depot.

### Characteristics of the organism

*Morphology. Streptococcus* (4–5 cells to a chain) when grown in litmus milk 48 hours at 37° C.

*Milk-agar plate.* Small circular colonies, less than 0·5 mm. in diameter.

*Milk-agar stab.* Growth along the line of inoculation. Very little surface growth.

*Litmus milk.* Firm acid clot in 24 hours at 37° C. without reduction of the litmus.

*Acid production.* Acid and no gas in fructose, dextrose, mannose, galactose, sucrose, maltose, lactose, glycerol and starch.

### Preliminary observations

In a preliminary test carried out on an aged litmus milk culture that had clotted at 37° C. and then been kept at room temperature for 5 days, it was found that on subculture (2 per cent. culture into litmus milk) only 0·02 per cent. of the cells survived pasteurization at 62·8° C. (145° F.) for 30 min., but, after 2 hours' incubation at 37° C., contrary to expectation, although the organisms had multiplied between three- and fourfold, the resistance to pasteurization had been increased from 0·02 to 30 per cent. After 4 hours' incubation the resistance had again dropped to a very small percentage.

In Table I the result of this preliminary test is recorded.

Table I

| Age of subculture hours at 37° C. | Bacteria per ml. | |
|---|---|---|
| | Unheated | Heated 62·8° C. for 30 sec. |
| 0 | 13,100,000 | 2,500 |
| 2 | 46,000,000 | 14,100,000 |
| 4 | 400,000,000 | 7,600 |

In view of the unexpected increase in resistance after the initiation of growth of the organism, it was decided to study its behaviour under different temperatures of growth.

### Experimental technique

Laboratory pasteurization was carried out in rubber-stoppered test-tubes completely immersed in a water bath at 62·8° C. (±0·25° C.) for 35 min.

Culture 11 was cultivated in sterilized litmus milk at 37° C. for 16–18 hours before each test. After incubation the litmus milk culture was acid, but not sufficiently acid to clot the culture medium.

At each test 0·5 ml. of the litmus milk culture was inoculated into 400 ml. sterilized homogenized whole milk. The inoculated sterilized milk was then

shaken vigorously before being tubed in 10 ml. quantities and placed in a water bath at the temperature to be employed throughout incubation. At varying intervals a tube of the sterilized milk culture was taken from the incubation bath and subjected to laboratory pasteurization. As each tube was transferred to a bath at 62·8°C. for this purpose, a control tube from the incubation water bath was plated out on milk enriched standard agar (standard agar + 0·5 per cent. milk), it having been shown in our previous paper (15) that this forms a satisfactory medium for the growth of the lactic streptococci. After heating, the culture was rapidly cooled to below 20°C. with cold water and immediately plated on milk agar. All plates were incubated in a water-jacketed incubator at 37°C. for from 40 to 48 hours, it also having been proved that maximum development of numbers was reached in this time, further incubation up to 5 days having no effect on the count of the test organism. The inoculated sterilized milk contained an approximately constant number of organisms—between one and two million per ml. before incubation.

*Resistance of culture* 11 *when incubated at* 42, 37, 30 *and* 26° C.

In Table II are recorded the average results of a number of tests made at 42, 37, 30 and 26°C. From these records it is seen that the resistance of culture 11 when grown at its optimum temperature (42°C.) appears to follow the accepted observation of decrease in resistance on the initiation of growth. As the growth rate of the organism is slowed down by cultivation at lower temperatures there is a tendency for the resistance of the growing culture to increase. At the lower growth temperatures an increase in resistance is recorded immediately prior to the initiation of growth and in the early stages of the logarithmic phase.

Table II. *Growth rate and heat resistance of culture* 11

| Time incubated | | 42° C. (3 tests) | | 37° C. (16 tests) | | 30° C. (5 tests) | | 26° C. (17 tests) | |
|---|---|---|---|---|---|---|---|---|---|
| hr. | min. | U. | H. | U. | H. | U. | H. | U. | H. |
| 0 | 0 | 6·1380 | 3·0153 | 6·0972 | 2·4905 | 6·1106 | 2·9935 | 6·0430 | 2·8540 |
| 0 | 10 | — | 3·1631 | — | — | — | — | — | — |
| 0 | 20 | 6·0590 | 2·9108 | 6·1705 | 3·9209 | — | — | — | — |
| 0 | 30 | 6·1399 | 2·1640 | — | — | — | — | — | — |
| 0 | 40 | 6·3365 | 1·9225 | 6·1948 | 3·4424 | — | — | — | — |
| 1 | 0 | 6·3876 | 1·5000 | 6·3043 | 3·2164 | 6·1274 | 4·0270 | 6·0828 | 4·8499 |
| 1 | 20 | — | — | 6·5559 | 2·9610 | — | — | — | — |
| 1 | 40 | — | — | 6·6067 | 3·4653 | — | — | — | — |
| 2 | 0 | 7·1874 | 0·5000 | 6·8884 | 2·3739 | 6·2863 | 3·4292 | 6·1777 | 3·9397 |
| 2 | 20 | — | 0·0000 | 7·1788 | 1·9430 | — | — | — | — |
| 3 | 0 | 8·0492 | — | 7·6712 | 1·8345 | 6·6005 | 4·6994 | 6·4036 | 4·4860 |
| 4 | 0 | — | — | 8·0098 | 0·3702 | 6·9955 | 3·0507 | 6·7173 | 4·3005 |
| 5 | 0 | — | — | 8·5007 | 0·5075 | 7·9092 | 1·4111 | 7·0660 | 2·1041 |
| 6 | 0 | — | — | 8·5375 | 1·3939 | 8·6512 | 1·4886 | 7·4324 | 1·2108 |

U. = unheated. H. = heated.

All figures represent averages of the logarithms of plate counts.

*Resistance of culture* 11 *when incubated at* 22° C.

The phenomenon is most marked at a growth temperature of 22° C., and in Table III are recorded the results of twelve tests made at this temperature.

For the first 3 hours' incubation at 22° C. very little growth was recorded; this period was followed by a period of from 1 to 3 hours' slow growth which was followed by the phase of most rapid growth at between the fourth and sixth hour after incubation had commenced.

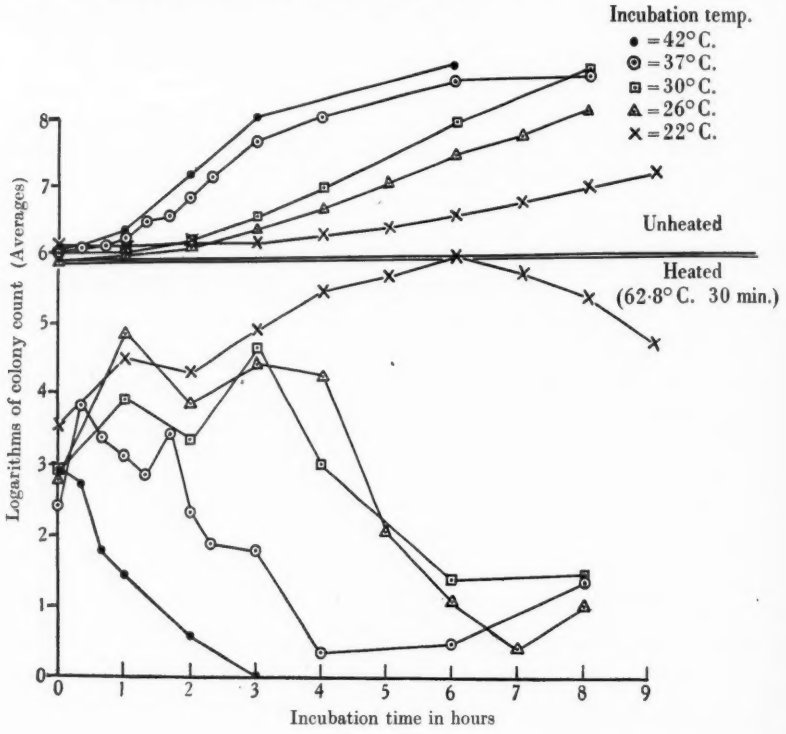


Fig. 1.

Throughout the phase of stationary and very slow growth there was a progressive increase in the total number of heat-resisting organisms. This increase continued until the phase of most rapid growth was well established, there being not only an increase in numbers found at this stage, but frequently an actual increase in the percentage of organisms surviving pasteurization.

In Fig. 1 the averages of the logarithms from Tables II and III have been plotted in the form of a graph.

In a recent publication by Mattick & Nichols(16) it is suggested that small changes in the *p*H of milk have a marked effect on the thermal resistance of the organisms present. Hourly tests made on culture 11 under similar

**Table III.** *Growth rate and heat resistance of culture* 11 (22° C.)

| Hours at 22° C. | Exp. 1 | | Exp. 2 | | Exp. 3 | | Exp. 4 | | Exp. 5 | | Exp. 6 | | Exp. 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U. | H. | U. | H. | U. | H. | U. | H. | U. | H. | U. | H. | U. | H. |
| 0 | 6·4065 | 1·6021 | 6·1584 | 3·8195 | 6·1399 | 2·1461 | 6·2148 | 1·6990 | 6·2695 | 4·6149 | 6·1790 | 4·6021 | 6·0128 | 4·7243 |
| 1 | 6·4265 | 1·6990 | 6·0374 | 5·6902 | 6·0792 | 3·7634 | 6·2279 | 4·0128 | 6·2967 | 5·7559 | 6·0792 | 3·9031 | 6·1139 | 5·6021 |
| 2 | 6·3892 | 0·0000 | 6·0414 | 5·6990 | 6·1399 | 3·4314 | 6·3541 | 3·4099 | 6·1931 | 6·0719 | 6·1430 | 4·2304 | 6·1367 | 5·9243 |
| 3 | 6·3820 | 3·2253 | 6·2148 | 5·8451 | 6·0792 | 1·9031 | 6·3032 | 3·5105 | 6·3617 | 6·3263 | 6·1072 | 5·2355 | 6·0414 | 5·9685 |
| 4 | 6·4150 | 3·4265 | 6·1644 | 5·8692 | 6·1931 | 5·6128 | 6·3579 | 4·6848 | 6·4771 | 6·3636 | 6·2122 | — | 6·1106 | 5·8921 |
| 5 | 6·4914 | 4·0334 | 6·2504 | 6·0719 | 6·2810 | 5·6232 | 6·5798 | 5·8388 | 6·7160 | 6·5933 | 6·1875 | 5·3560 | 6·3054 | 6·0334 |
| 6 | 6·5955 | 5·7634 | 6·3181 | — | 6·4624 | 6·0569 | 6·7924 | 6·3838 | 6·8808 | 6·3201 | 6·4314 | 5·4048 | 6·5051 | 6·1614 |
| 7 | 6·7993 | 4·6628 | 6·5752 | 6·2041 | 6·6128 | 5·9823 | 6·9685 | 3·2923 | 7·0414 | 6·4031 | — | — | 6·6590 | 6·4200 |
| 8 | — | — | — | — | — | — | 7·1367 | 3·2553 | 7·1004 | 6·1553 | 6·7709 | 5·1818 | 7·0453 | 5·4183 |
| 9 | — | — | — | — | — | — | 7·4116 | 2·5563 | 7·3444 | 3·7634 | 7·1399 | 4·6435 | 7·0828 | 5·4955 |

| Hours at 22° C. | Exp. 8 | | Exp. 9 | | Exp. 10 | | Exp. 11 | | Exp. 12 | | Averages of logs. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U. | H. | U. | H. | U. | H. | U. | H. | U. | H. | U. | H. |
| 0 | 6·2122 | 4·2765 | 6·2601 | 4·4183 | 6·2455 | 3·6435 | 6·2625 | 4·3010 | 6·3054 | 3·8062 | 6·2222 | 3·6378 |
| 1 | 6·1271 | 4·8808 | 6·3243 | 5·5775 | 6·2148 | 4·3617 | 6·2625 | 5·5315 | 6·2718 | 4·1004 | 6·2051 | 4·5732 |
| 2 | 6·2148 | 5·1553 | 6·2765 | — | 6·2967 | 4·1523 | 6·2553 | 5·8573 | — | — | 6·2219 | 4·3932 |
| 3 | 6·2405 | 4·8808 | 6·2148 | 5·7160 | 6·2625 | 5·3483 | 6·2601 | 5·9685 | 6·2923 | 5·6435 | 6·2299 | 4·9643 |
| 4 | 6·2355 | 5·5051 | 6·3385 | 5·8692 | 6·3802 | — | 6·3838 | 6·0607 | 6·4609 | 5·8388 | 6·3107 | 5·5123 |
| 5 | 6·3674 | 5·7993 | 6·3747 | 5·9590 | 6·4393 | — | 6·5809 | 5·9956 | — | — | 6·4158 | 5·7304 |
| 6 | 6·4014 | — | 6·5705 | 6·4487 | 6·4786 | 5·8921 | 6·7559 | — | 6·7709 | 5·6128 | 6·5802 | 6·0049 |
| 7 | 6·6721 | 6·1761 | 6·8129 | 6·0374 | 6·6857 | 5·9823 | 6·7924 | 6·4330 | — | — | 6·7619 | 5·7593 |
| 8 | 6·7482 | 6·4281 | 6·9638 | 5·3766 | 6·9542 | 5·0492 | — | — | 6·9777 | 6·4472 | 6·9621 | 5·4140 |
| 9 | 6·9294 | 6·5198 | 7·0682 | 5·6294 | — | — | 7·1106 | 5·0000 | 7·2923 | 4·4031 | 7·1724 | 4·7514 |

U. = unheated culture. H. = culture heated at 62·8° C. for 30 min. All colony counts expressed as logarithms.

conditions to those described in the reported tests failed to show any change in reaction in 4 hours at 37° C. or in 7 hours at 22° C.

The possible effect of transferring the culture from an acid to a neutral medium has also been considered. Although the addition of 0·5 ml. of the culture in an acid medium to 400 ml. of sterilized milk did not alter the *p*H of the resulting culture medium, it was thought possible that an adsorbed acid layer on the test organisms or even a change in cell structure might result from the prolonged association of the cells with the original acid inoculum. In order to test this point, culture 11 was grown in a non-sugar broth of 0·5 per cent. tryptone (Difco) and 0·1 per cent. peptone (Fairchilds) to which had been added 0·5 per cent. A.R. calcium carbonate. This medium after inoculation with culture 11 was kept in gentle motion throughout the 18-hour incubation period at 37° C., and a final culture of *p*H 7·0 was obtained for inoculation into the test sterilized milk. It is inconceivable that under these conditions any adsorbed acid layer could exist.

The results of two broth culture tests are recorded in Table IV.

Table IV. *Resistance of broth to milk culture* 11

| Culture age 30° C. | Exp. 1 | | Exp. 2 | |
|---|---|---|---|---|
| | U. | P. | U. | P. |
| 0 | 4·5911 | 0·0000 | 4·2041 | 0·0000 |
| 1 | 4·7243 | — | 4·4771 | 2·3222 |
| 2 | 4·8261 | 3·5065 | 4·6990 | 3·7243 |
| 3 | 4·9542 | 3·4871 | 5·1703 | 3·4771 |
| 4 | 5·4314 | 2·2304 | 5·5441 | 3·5315 |
| 5 | — | 2·8751 | 6·2175 | 3·3404 |

U. = unheated culture. P. = culture heated to 62·8° C. for 30 min.

All plate counts expressed as logarithms.

From the figures it is seen that the non-sugar broth was not such a suitable medium as milk in which to grow culture 11. The plate count of the 18-hour broth culture was in the neighbourhood of 20 millions per ml. against the 500 millions per ml. in an 18-hour milk culture.

No increase in count was found after a further 8 hours' incubation of the broth culture.

On incubation at 30° C. of sterilized milk after inoculation with the broth cultures a gradual increase in resistance was again observed on the initiation of growth.

*Effect of refrigeration on heat resistance*

As culture 11 appeared to be weakening in its characteristic resisting properties, further cultures of resistant streptococci were isolated from laboratory pasteurized commercial milk supplies. Of forty isolated cultures six showed similar heat-resisting properties to those of culture 11, and one (culture U 2) was selected for further study.

Besides repeating the experiments on growth rate and heat resistance, a number of experiments were made in which the incubated milk was pasteurized immediately after incubation, and also a second test was made by

pasteurizing the incubated milk after cold storage at 3–4°C. for 24 hours. The results of these combined tests are recorded in Table V, and in Table VI the results from similar experiments on culture 11 are shown.

From the results recorded in Table V it is seen that culture U 2 shows a similar increase in heat resistance on the initiation of growth to that of culture 11. The increase is again most marked and of longer duration as the rate of growth of the organism is slowed down.

The effect of 24 hours' storage at 3–4°C. on both cultures 11 and U 2 is marked. When refrigeration is applied during the early stages of growth the refrigerated culture shows a considerably higher percentage survival after heating than does the non-refrigerated culture. At the same time the increased resistance of the refrigerated culture tends to continue farther into the logarithmic phase of growth.

## SUMMARY AND DISCUSSION

A study of the graph reproduced in Fig. 1 shows clearly that while the organism under discussion follows the normal growth and resistance curve when cultivated at or near its optimum temperature, as the rate of growth is slowed down by lowering of the incubation temperature, this resistance curve is considerably modified. Previous workers (Sherman & Cameron (11,12); Rogers (17)) have already pointed out that the slowing down of growth results in a more hardy organism, but, so far as our study of the literature has gone, we have not seen any recorded instance of sluggish growth resulting in a progressive increase in resistance throughout the lag and early logarithmic phases of growth.

This phenomenon, which we have observed in other cultures of streptococci with an optimum growth temperature in the neighbourhood of 37°C., may have considerable importance in the handling of commercial milk supplies. A supply of uncooled raw milk infected with these organisms, held during several hours at summer temperature, might develop a resistant flora by the time it reached the cooling station.

It is probable that Stark (10) in overcoming a ropy milk outbreak by incubation of milk at 26–30°C. was working very close to the optimum growth temperature of the ropy milk organism, at which temperature, as in the case of culture 11, there would be no preliminary increased resistance.

The most common temperatures used in the storage of raw milk before pasteurization lie between 3 and 7°C. The figures that are presented on the question of refrigeration suggest the possibility of increased resistance in raw milk supplies as a result of brine cooling bulk supplies of milk in the country before despatch to the town pasteurizing depots.

It has been observed in certain commercial supplies cooled to this temperature at the receiving stations and transported to the city in insulated tanks, that the resistant count at the former is frequently much lower than at the latter.

## Table V. *Heat resistance of culture U 2 before and after cold storage at 3–4° C. for 24 hours*

| Culture age 37° C. hours | Exp. 1 | | | Exp. 2 | | | Exp. 3 | | | Exp. 4 | | | Exp. 5 | | | Exp. 6 | | | Averages of logs. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. |
| 0 | 5·9243 | 3·0682 | 3·0453 | 5·6812 | 2·0792 | 3·5798 | 5·4771 | 4·6096 | 4·1206 | 5·7482 | 4·1761 | 5·2014 | 5·5315 | 4·3979 | 3·7853 | 5·4314 | 4·9542 | 5·0000 | 5·6323 | 3·8808 | 4·1254 |
| ½ | 5·9542 | 3·2788 | 4·9191 | 5·6435 | 3·0969 | 4·9542 | 5·5658 | 4·7853 | 5·3404 | — | — | — | — | — | — | — | — | — | 5·7212 | 3·7203 | 5·0712 |
| 1 | 6·0569 | 3·9243 | 5·1367 | 5·7924 | 4·0212 | 5·3502 | 5·6990 | 4·9445 | 5·8513 | 5·7993 | 4·9542 | 5·7324 | 5·7853 | 4·5198 | 5·6232 | 5·6812 | 5·7243 | 5·7324 | 5·8023 | 4·6814 | 5·5710 |
| 1½ | 6·2504 | 4·6232 | 5·2380 | 5·8195 | 4·7709 | 5·7782 | 6·0792 | 4·8325 | 5·9085 | — | — | — | — | — | — | — | — | — | 6·0497 | 4·7422 | 5·6415 |
| 2 | 6·5051 | 2·6335 | 4·9243 | 6·2068 | 4·0000 | 5·9031 | 6·2648 | 5·0719 | 5·8129 | 6·0645 | 4·1335 | 6·0645 | 6·3139 | 4·7404 | 5·9685 | 6·2900 | 3·7924 | 6·0128 | 6·2742 | 4·0619 | 5·7810 |
| 3 | — | — | — | — | — | — | — | — | — | 6·8808 | 3·8451 | 5·2455 | 6·8865 | 3·3802 | 3·8062 | — | 1·3010 | — | 6·8836 | 2·8421 | 4·5258 |
| 4 | — | — | — | — | — | — | — | — | — | 7·4314 | 2·0414 | 0·0000 | 7·3404 | 1·0000 | 0·0000 | 7·3222 | 3·0043 | 1·0000 | 7·3647 | 2·0152 | 0·3333 |
| 5 | 8·0755 | 0·0000 | 3·1461 | 7·5563 | 1·6990 | 0·0000 | 7·7243 | 3·3820 | 0·0000 | 7·7160 | 0·0000 | 2·3424 | 7·6232 | 0·0000 | 1·4771 | 7·7404 | 1·4770 | 1·3010 | 7·7393 | 1·0930 | 1·3777 |

| Culture age 22° C. hours | Exp. 4 | | | Exp. 5 | | | Exp. 6 | | | Averages of logs. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. |
| 0 | — | 4·1761 | 5·2014 | 5·5315 | 4·3979 | 3·7853 | 5·4314 | 4·9542 | 5·0000 | 5·4814 | 4·5094 | 4·6622 |
| 1 | 5·7160 | 4·8451 | 4·8325 | 5·6628 | 4·6721 | 4·6128 | 5·4314 | 5·4150 | 5·3424 | 5·6034 | 4·9774 | 4·9292 |
| 2 | 5·5911 | — | 5·5441 | 5·6128 | 5·0569 | 5·1303 | 5·6128 | 5·4771 | 5·4771 | 5·6056 | 5·2670 | 5·3838 |
| 3 | 5·7559 | 5·4771 | 5·5911 | 5·7243 | 5·2945 | 5·7404 | — | 5·1239 | — | 5·7401 | 5·2985 | 5·6657 |
| 4 | 5·7993 | 5·7782 | 5·7482 | 5·8513 | 5·7559 | 5·9777 | 5·5911 | 5·5315 | 5·6535 | 5·7472 | 5·6885 | 5·7931 |
| 5 | 6·0414 | 5·3201 | 5·9294 | 5·9542 | 5·3560 | 6·0531 | 5·8513 | 5·4065 | 5·7482 | 5·9489 | 5·3608 | 5·9102 |
| 6 | 6·1614 | 4·4698 | 6·1903 | 6·2480 | 5·8976 | 6·1303 | 6·0170 | 5·3729 | 6·0043 | 6·1421 | 5·2468 | 6·1083 |

U. =untreated culture.
P. =culture heated to 62·8° C. for 30 min. without previous cold storage.
R.P. =culture heated to 62·8° C. for 30 min. after cold storage.

## Table VI. *Refrigeration and heat resistance of culture* 11

| Culture age 30° C. | Milk 1 | | | Milk 2 | | | Broth 1* | | | Broth 2* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. | U. | P. | R.P. |
| 0 | 5·8573 | 4·5051 | 5·4683 | 5·6232 | 4·3617 | 4·1761 | 4·5911 | 0·0000 | 2·8751 | 4·2041 | 0·0000 | 1·3010 |
| 1 | 5·8692 | 5·1206 | 5·7782 | 5·6990 | 4·0792 | 4·8921 | 4·7243 | — | 2·5798 | 4·4771 | 2·3222 | 3·3979 |
| 2 | 5·9868 | 4·5563 | 6·0414 | 6·1847 | 2·8865 | 5·4314 | 4·8261 | 3·5065 | 4·1790 | 4·6990 | 3·7243 | 4·6721 |
| 3 | 6·8388 | 5·2577 | 5·6435 | 6·7076 | 3·0755 | 5·7782 | 4·9542 | 3·4871 | 4·5441 | 5·1703 | 3·4771 | 4·9638 |
| 4 | 7·3010 | 4·2833 | 6·3096 | 7·3010 | 3·0755 | 4·7782 | 5·4314 | 2·2304 | 5·0043 | 5·5441 | 3·5315 | 5·3424 |
| 5 | 7·5563 | — | 5·7404 | — | — | — | — | 2·8751 | 4·9823 | 6·2175 | 3·3404 | 5·7782 |

U. =unheated sterilized milk culture.
P. =culture pasteurized at once.
R.P. =culture pasteurized after 24 hours at 3–4° C.
All plate counts expressed as logarithms.
* Original culture grown in broth + A.R. $CaCO_3$ for 18 hours at 37° C. 1 ml. of broth culture was transferred to 1000 ml. of sterilized milk for these tests.

While it is admitted that the plate count of streptococci may be subject to certain variations that would not be encountered with some other organisms, the even growth curves of the unheated cultures at all temperatures and the numerous experiments showing the same phenomenon prove, in our opinion, that the technique adopted in these experiments is satisfactory.

It is possible that all the cells pass through a resistant stage but do not reach this resistant stage at the same time, and that their period of existence in this stage depends on the temperature. The higher this is up to the optimum, the shorter this stage will be. If this assumption is correct, the slower the rate of growth the greater the percentage of organisms which will be found in the resistant stage at one and the same time during the early growth phase. This theory agrees with the observed facts. The experiments recorded in this paper have been made on two organisms only, one of which has now, as a result of subculturing over years, lost its character.

## CONCLUSIONS

1. A *Streptococcus* has been described which shows increased resistance to heat as its temperature of growth is reduced below the optimum.

2. At temperatures below the optimum for growth, the culture described shows a progressive increase in resistance during the early logarithmic growth phase.

3. Two cultures of streptococci are shown to increase in resistance as a result of 24 hours' cold storage.

4. The increase in resistance as a result of cold storage appears to be most marked if the low temperature is applied during the early logarithmic phase of growth, particularly when applied to a slowly growing culture.

5. Whether this phenomenon is shown by members of the streptococci other than those related to *Streptococcus thermophilus* is not known.

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# BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

## SECTION D[1]. THE NUTRITIONAL VALUE OF MILK AND MILK PRODUCTS


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C. Milk products.
(*a*) Cheese.


### INTRODUCTION

THE present review deals mainly with papers published between the spring of 1934 and the beginning of 1936. It is chiefly devoted to liquid cow's milk, but the nutritional value of various milk products has been considered and, in this case, the

[1] Formerly Section E.

literature from 1932 has been consulted. As in the previous review (1), wherever possible all the papers have been consulted in the original. Those inaccessible and known only from abstracts are marked with an asterisk in the bibliography.

## GENERAL

The second edition of the *Fundamentals of Dairy Science* (2) contains an up-to-date chapter on the nutritional value of milk and milk products, and much useful information will be found in the recent book on dairy chemistry by Davies (3). The report by Hiscock (4) should be consulted. An interesting, though popular discussion of the value of milk as food will be found in a book by Angelici (5).

Judging by the number of publications the two subjects mentioned in the previous review (1), namely, the effect of heat on milk and the methods of increasing the content of certain factors in milk, still attract a good deal of attention.

## COW'S MILK

### I. Nutritional value as estimated in laboratory experiments

#### A. *Raw milk*

(*a*) *General.*

Seasonal differences in the total nutritional value of milk have been noticed by Elvehjem *et al.* (6), who fed rats on an exclusive diet of milk supplemented with iron, copper and manganese. More rapid growth was noticed with autumn than with winter (December or February) milk. The authors suggest that the difference may be due to changes in the vitamin $B_4$ content of milk, but it must be said that the existence of this vitamin is problematic (7). As the intakes of milk are not given it is not known whether the differences are due to changes in appetite or in the utilization of the milk. According to Peterson *et al.* (8), who used the same technique as in (6), milk produced on A.I.V. silage is superior to winter milk. Similar seasonal differences in the nutritional value of sera prepared from skimmed milk by treatment with rennet and subsequent heating have been observed by Stirn *et al.* (9).

Toxic amounts of fluorine when fed to cows have no effect on the nutritive properties of the milk for rats (10).

The nutritive importance of milk has been strikingly demonstrated in prolonged experiments by Orr *et al.* (11), who fed to rats a diet based on a dietary survey of an industrial population, and showed that it could be much improved by addition of milk and vegetables.

Keil & Nelson (12) have further investigated the sterility of rats receiving whole milk supplemented with iron and copper (the earlier work has been discussed in the previous review). They have come to the conclusion that the sterility is not due to degeneration of the sex organs, since males were found to produce motile spermatozoa and gave positive matings with stock females, while females produced normal litters after their diet had been changed to the stock diet.

(*b*) *Vitamins.*

This subject is still as popular as ever, and many papers dealing with it have been published. It is gratifying to note that increasing use has been made of international vitamin standards and that, in some cases at least, it is possible to submit results of different workers to more quantitative comparisons. There is still much room for improvement of methods and their applications, and many points will have to be cleared up before general agreement is reached. The highest observed values for vitamin A have been given by one laboratory (13) as less than 20 international units per gram for pasture butter and by another (14) as 200 units for an

unspecified butter. Such widely different results obtained by two distinguished students of nutrition certainly arouse doubts as to the accuracy of the application of existing methods. A similar instance in the case of vitamin D is found on p. 196. In addition to the books and general summaries already mentioned, surveys of the vitamins of milk will also be found in papers (15) and (16). Paper (17) gives a useful summary of vitamins A and C in milk.

(i) *Vitamin A.* Of the papers read by the reviewer, those on vitamin A and its precursor carotene, claim more than the rest of the vitamins together. The majority deal, in one form or another, with the transfer of carotene from feed to milk and its partial conversion into vitamin A. This biochemical process is so well known that its serious misrepresentation (18) comes as a real surprise.

The physical estimation of carotene in butter is still causing some trouble. In spectrophotometric measurements on butter itself, opinions as to the applicability of Beer's Law are divided (19, 20). When, for more exact measurements, butter is saponified before the spectrophotometric test is applied, difficulties of a different nature are encountered. One of them is that carotene in solutions of the non-saponifiable residue is more unstable than vitamin A (21). Another peculiar difficulty is linked with the estimation of the various isomeric forms of carotene. According to Kuhn *et al.* (22) the $\beta$-isomeride is biologically twice as active as the $\alpha$ and $\gamma$ varieties. To be of biological significance the spectrophotometric estimation of carotene in butter must be qualified by the determination of which isomeride (or isomerides) is present in butter and in what proportions. This is done by chromatographic examination (23), which according to Gillam & el Ridi (24) can easily lead to fallacious results.

The addition of artificial colouring matter to butter is condemned in this country (25). In Germany, on the other hand, the more stable chemical dyes are favoured (26, 27) in preference to carotene, on the rather doubtful ground that even winter butter contains sufficient vitamin A. The relative biological efficiency of the vitamin A and carotene of butter has been investigated by Booth *et al.* (28), who estimate that vitamin A in butter is six times as potent as carotene, though they admit that their indirect calculations may be subject to appreciable error. It is interesting to note in this respect that Kraybill & Shrewsbury (29) have recently reported that carotene dissolved in butter which had been completely decolorized and freed from vitamin A by treatment with Lloyd's reagent is 2–3 times less potent biologically than the same amount of carotene dissolved in cotton-seed oil. The stability of the carotene in butter had been checked by spectrophotometric methods.

Very interesting observations on the colour of butters churned at the October Dairy Show (Agricultural Hall, London) in the course of a decade have been recently reported by Whetham & Hammond (30). Observations on the seasonal variations of the colour of Danish butters will be found in a paper by Knudsen & Nielsen (31).

Numerous reports have been published on the effect of feeding cows with A.I.V. silage on the vitamin A and carotene content of their milk (or butter) (32, 8, 33, 34, 35, 13). All workers are agreed that this method of increasing the vitamin A activity of winter milk is highly satisfactory, though not all share the view of the inventor of the fodder (33) that the milk is quite equal to summer milk in vitamin A and carotene content (8, 35). Good results are also reported after feeding ordinary silage (36), alfalfa hay (especially artificially dried (37, 38)), and artificially dried grass (35). The high intakes of carotene on such rations are recovered in the milk to only a small extent, generally not more than 3·5 per cent. (8, 38, 39).

Much higher percentage recoveries (up to 87 per cent.) have been reported by Fraps *et al.* (40) on low intakes of carotene. But it is possible that in this case the cows were drawing on their body reserves. On equally low intakes Copeland &

Fraps(41) had previously reported a retention figure of only 2 per cent., and it should be remembered that calculations of balances are somewhat complicated by the fact that normally the cow ingests carotene only but secretes carotene and vitamin A.

The calculations in papers(38, 40, 41) are based on biological estimations, those of paper (8) are partly and those of paper(39) entirely derived from physical measurements.

It is generally agreed that when either silage or artificially dried grass is fed in sufficiently large amounts "ceiling" values for carotene and vitamin A activity of the butter are reached. These values fail to increase on further additions to the fodder(37, 36, 35), though some increase may take place when the cows are turned out to pasture.

Hilton *et al.*(37) and Wilbur *et al.*(42) report the very interesting observation that soya beans, either roasted or raw, when added to the fodder, interfere with the assimilation of carotene and lower the vitamin A content of butter.

Booth *et al.*(28) found no difference in vitamin A activity between samples of winter, summer and autumn Guernsey butter, and similar samples of Shorthorn butter produced under identical conditions. The former butters contained at all seasons more carotene and less vitamin A than the Shorthorn butters. Similar observations for Holsteins, Ayrshires and Guernseys are reported by Beeson(43). It would follow that in these breeds the milks richest in fat would be most potent in vitamin A. On the other hand, Sutton & Krauss(44) report statistically significant differences in favour of Holsteins and Guernseys as against Ayrshires and Jerseys when the respective butters produced under similar conditions were tested biologically for vitamin A. These authors also noted the breed differences in the relative carotene and vitamin A contents of the butters.

Semb *et al.*(45) have confirmed the original findings of Drummond *et al.*(46), and the recent observations of Dann(47) that colostrum is a much richer source of vitamin A and carotene than normal milk. According to these authors(45) the blood stream of the cow can function as an important storage place for carotene.

It is reported by Gillam & el Ridi(48) that in winter and in summer cow blood sera contain several times more carotene and vitamin A than the corresponding sera from bulls. In view of the known differences in feeding it is impossible to say whether the difference is of nutritional or sex origin.

Measurements of the vitamin A activity of milk at different seasons will be found in papers (49, 50, 17, 51). The latter three papers give also carotene and vitamin A (Lovibond) values.

(ii) *The vitamin B complex.* Only few papers have been published on this subject. Dutcher *et al.*(52) have studied the vitamin $B_1$ and $B_2$ content of milk.

The discovery of Kuhn and his school relating to the vitamin $B_2$ activity of lactoflavin has been reported in the previous review(1), but it seems at present that the relationship is not as simple as it was formerly believed. A good discussion of the role of lactoflavin in relation to other components of the vitamin B complex may be found in a recent paper by Birch *et al.*(53). Free lactoflavin, but not its ester, is present in the milk(54).

(iii) *Vitamin C.* The chemical estimation of vitamin C in milk by titration with 2-6-dichlorophenol indophenol shows that the level is independent of the season(50, 17, 51, 55) and of the ration(55). This last paper also gives similar results of biological tests. Peculiar changes associated with the season but not with changes in nutrition are reported by Rohmer *et al.*(56). A further study of the effect of light on the ascorbic acid of milk(1) shows that at first the oxidation is largely reversible, but longer exposures bring about irreversible oxidation(57). It appears that light starts

a chain reaction, and that oxidation once started proceeds in darkness as well (58). Titration figures for raw milk in Milan are given by de Caro & Speier (59). The values are very low, but as the method of titration used would not estimate reversibly oxidized ascorbic acid the values cannot be interpreted. The same applies to the findings of Jacobsen (60). A few figures for vitamin C in milk obtained in biological tests will be found in a paper by Fellers (61).

(iv) *Vitamin D.* Batchelder (62) gives some vitamin D values for milk and butter. The vitamin D content of Dutch butter at different seasons will be found in (50) and also in (63). According to Coward & Morgan (14), the vitamin D content of seventeen samples of dairy butter varied from 0·4 to 4 international units per gram, with an average of 1·2 units. These figures are surprising, as in numerous tests carried out on south of England butters the reviewer has never obtained for best pasture butters values higher than 0·7 international unit per gram (64). Fellers (61) also gives figures for vitamin D in milk.

(*c*) *Other factors.*

(i) *Lactose.* The nutritional function of the characteristic sugar of milk has occupied the minds of research workers for some time, and recent observations have yielded interesting results. Lactose, when added in sufficient amounts to rachitogenic diets, has been found to exhibit definite antirachitic effects in the case of chicken (65) and rats (66).

It is probable that this effect is due to increased acidity of the intestinal tract and better absorption of the bone-forming elements. Lactose is in general poorly utilized by adult animals; a review of the relevant literature will be found in a paper by Koehler & Allen (67). Their own experiments lead these authors to state that from 40 to 50 per cent. of the ingested lactose may be lost to the rat as far as weight and energy relationships are concerned. The acidifying action of lactose is caused by fermentation of the unabsorbed sugar.

The notable finding has been reported from different laboratories that the inclusion of large amounts of lactose in experimental diets brings about the formation of cataracts in rats (68, 69). The onset of the symptoms is roughly parallel to the concentration of lactose in the diet. Thus a diet containing 70 per cent. of lactose produced a mature cataract in some 10 weeks in 70 per cent. of the experimental animals. The incidence is smaller (30 per cent.) and the time of incubation longer (17 weeks) on a diet containing 50 per cent. of lactose. No cataract developed on a diet containing 30 per cent., but it is noteworthy that some changes in the lens could be detected even on the lowest level in practically all rats, after a comparatively short period on the diet. As would be expected, galactose produces the same effect (70, 71, 72). The action of galactose is very rapid, and mature cataracts may develop within a fortnight. Rabbits have been found to be refractory to lactose feeding and developed no lens abnormalities on a 70 per cent. lactose diet (68). Though it is obvious that lactose and galactose interfere in some way with the metabolism of the lens, the mechanism of this action is not yet understood, though it has been suggested (69) that as rats receiving lactose are hyperglycaemic the mechanism may be the same as with diabetic cataract. Clinical descriptions of the development of the nutritional cataract are given in papers (68) and (72). The histopathology is given in paper (73).

Rats receiving an exclusive diet of cow's milk supplemented by iron, copper and manganese, consume in the solids some 40 per cent. of lactose. Rats have been kept for five generations on such diets (74), but no lens degeneration has ever been mentioned in the numerous reports on rats receiving mineralized milk, and it is questionable whether lactose as present in milk is as toxic as the isolated substance.

An ophthalmoscopic examination of twenty-four rats which had been kept in the reviewer's laboratory for 180 days on an exclusive diet of milk supplemented with Fe, Cu and Mn, showed no abnormalities (75). Whittier *et al.* (76) have found that rats receiving lactose contained less fat than animals receiving similar quantities of sucrose. Animals fed on lactose were found to live longer than their litter mates on the sucrose diet.

It is mentioned in the *7th Biennial Report of the Kansas Agricultural Experiment Station* (77) that the brains of rats receiving 20 per cent. of lactose in their diet contained much more cerebrosides than the brains of rats receiving a similar diet with sucrose, and it is stated that a significant correlation was found between "learning ability and both unsaturated cerebrosides and unsaturated phosphatides".

(ii) *Soft-curd milk, natural and artificial.* The interest in soft-curd milk seems to be increasing in the United States of America and in Canada, where the milk is becoming a commercial proposition. Good reviews on natural and artificial soft-curd milk (such as is produced by heating, homogenization or base exchange) will be found in papers by Theophilus (78), Berry (79) and especially Doan & Welch (80). The manufacture of soft-curd milk by base exchange is described by Otting & Quilligan (81). The value of soft-curd milk in human nutrition is discussed in a following section.

The results of experiments on animals need careful consideration because soft-curd milk is usually low in casein, and although certain normal healthy cows do produce soft-curd milk it is generally secreted in cases of mastitis.

(iii) *Milk minerals.* Experimental milk anaemia is discussed in the comprehensive review of Davidson & Leitch (82). The essential nature of manganese and its deficiency in cow's milk is strikingly demonstrated by Daniels & Everson (74). In their experience the very high mortality of young rats produced by mothers receiving a milk-iron-copper diet is not relieved by transferring the young to foster mothers on a normal stock diet. The mothers secrete apparently satisfactory milk and are able to suckle normal young successfully, but their own succumb to congenital debility caused by lack of manganese *in utero*. Porter (83) has shown that milk anaemia predisposes rats to infestation with *Nippostrongylus Muris*, and that the addition of iron and copper does not raise the resistance to the level imparted by a stock diet.

(iv) *Milk proteins.* For the proteins of raw liquid milk (skimmed) Fairbanks & Mitchell (84) give a true digestibility of 95 and a biological value of 90 at a nitrogen level of about 8 per cent.

### B. *Treated milk*

(*a*) *Heated milk.*

(i) *General.* No extensive surveys of the effect of heat treatment on milk are known to the reviewer, but a useful summary of the literature will be found in the bulletin of M'Candlish & Black (85), and also in a review by McHenry (86). A short critical article has been published by McCollum (87) who expresses the opinion, based on the work of others and his own experience, that processed (pasteurized, dried and evaporated) milks are nutritionally equivalent to raw. A very interesting collection of opinions of various authorities on the effect of pasteurization on the nutritive value of milk will be found in the *Scottish Journal of Agriculture* (88).

Of original papers the following are of interest. Paper (6), which has already been partly reviewed on p. 193, discusses differences in nutritional value between raw and pasteurized milk. The authors found that "season or the kind of feed ingested by the cow has a much greater effect upon the nutritive value of milk than does pasteurization.... Pasteurization has practically no detrimental effect as

measured with rats upon the nutritive value of milk of high nutritive quality, but may further decrease the nutritive value of milk of low nutritive quality". The technique of these tests has already been described, and the same remarks apply here as were made on p. 193. Lasby & Palmer (89) have found that milk pasteurized in the laboratory in glass vessels caused poorer growth and a higher degree of anaemia than raw milk from the same bulk, but the differences were not statistically significant. The milks were tested as sole food in paired feeding experiments on rats. When commercially pasteurized milk was compared with raw in similar experiments the results were reversed, and the difference in haemoglobin content in favour of rats on pasteurized milk was statistically significant; the difference in body weight although consistent was not. The superiority of commercially pasteurized milk was attributed to the gain of iron and copper in the process of pasteurization. Brannon & Prucha (90), using rats, have also compared milk before and after pasteurization given *ad lib.* as the sole diet, and found that rats on pasteurized milk did much better than those receiving raw. They suggest that the poor performance on raw milk was due to bacterial causes. No evidence is given to support this view, and, judging from the symptoms described, it is just as probable that the raw milk group was suffering from nutritional anaemia which might have been prevented in the other group by the pasteurized milk having picked up Fe and Cu in the process of manufacture. A similar nutritional inferiority of raw milk as compared with pasteurized and especially with flash pasteurized milk has been described by Hertel (91). The flash pasteurization (12 sec. at 76° C.) was carried out in a commercial plate heater. Holder pasteurization was carried out in the laboratory (30 min. at 63° C.), and the material from which the container was made is not stated. The experiments were complicated by infection with *B. enteritidis* Gärtner. After a preliminary period when the milks were fed alone, iron but not copper was added. Rats on raw milk lost most weight and died out after about 90 days, those on laboratory pasteurized milk died at the same time but lost less weight. The majority of those on the commercial flash pasteurized milk survived for 180 days and gained weight consistently. The explanation of the author, that the power of resistance of the rats receiving the raw milk was lowered by resorption of the unchanged proteins of milk and that they then succumbed to the *B. enteritidis* infection, can hardly be taken seriously. On the other hand, the possibility of a Cu-deficiency anaemia is by no means excluded.

Okada & Sano (92) have studied on mice the effect of heating milk at various temperatures and have found a progressive nutritional deterioration with increasing temperature. Cimmino (93) found no difference in the growth of rats on an exclusive diet of raw or pasteurized milks supplemented with iron. Whether the milks were from the same source is not stated.

The extent to which the nutritional value of milk is affected by pasteurization is still unsettled. New experiments on this problem are mentioned on p. 203.

(ii) *Vitamins.* According to (17) and (51) the vitamin A of milk is not affected by pasteurization or stassanization, but de Caro & Speier (59) think that it undergoes a slight loss, which they believe also occurs after 2 min. boiling. These authors found no effect of pasteurization or boiling on the vitamin B complex of milk. Dutcher *et al.* (52), however, found that all the following procedures resulted in some loss of the vitamin $B_1$ and $B_2$ of milk: (1) laboratory pasteurization in glass; (2) same under reduced pressure; (3) same under aeration; (4) boiling for 10 min. Boiling had the least effect and, surprisingly enough, pasteurization in partial vacuum was the most detrimental. It is to be regretted that the data are based on the performance of selected animals only.

In contrast with these findings, Davis & Norris (94) found that holding liquid

skimmed milk in storage tanks at 170° F. for up to 24 hours had no effect on the vitamin $B_2$ content as measured on chicks.

Some loss of vitamin C in the course of pasteurization is reported by all those who have studied the problem (50, 17, 51, 59, 61, 95, 96), though flash pasteurization (95) and stassanization (50, 17, 51) are stated to be almost without effect. No difference was found in the effect on vitamin C between ordinary laboratory pasteurization and pasteurization after the air had been removed from the milk *in vacuo* (96).

Vitamin D is not affected by pasteurization (61).

(iii) *Other factors.* Lasby & Palmer (89), in paired feeding experiments, have studied the metabolism of nitrogen, Ca and P of adult rats receiving an exclusive diet of either raw or laboratory pasteurized milk supplemented with Fe and Cu. No significant differences were found in the availability of these elements. By mistake, percentage excretions are given in the paper instead of percentage retentions, and a correction to the paper is about to be published (97).

(*b*) *Irradiated and vitaminized milk.*

In the course of the two years which have elapsed since the previous review (1) was written the problem of increasing the vitamin D content of milk has lost nothing of its popularity, and numerous papers have been published on the subject. Strictly speaking, milk normally produced by specially treated cows cannot be considered as treated milk, but it is much more convenient to discuss in this chapter milk secreted by cows receiving powerfully antirachitic substances.

The methods of increasing the vitamin D potency of dairy products have been ably summarized by Krauss & Bethke (98) and Palmer (99). Further discussions of the three main methods of achieving this purpose—feeding of irradiated yeast to cows, direct irradiation of milk and addition to it of a suitable concentrate—will be found in papers (100, 101, 102, 103, 104, 105). The last contains photographs and descriptions of various irradiation methods. A good historical survey is given by Friedman (106). The legal aspects in the United States are reviewed by Smith (107), and are discussed in (108). The labelling of the various antirachitic milks in the United States is discussed in (109). Warning notes against commercial enthusiasm are sounded in the report of the Milk and Dairy Products Committee of the American Public Health Association (110) which is supported by an Editorial in the *American Journal of Public Health* (111).

In original work, further investigations of the antirachitic activation of milk by ultra-violet irradiation have been carried out by Supplee and his collaborators (112, 113, 114, 115, 116, 117). It was found that the formation of vitamin D, which is almost instantaneous, takes place substantially at the surface of the milk. A film of milk 0·02 mm. thick transmits only about 30 per cent. of radiations of 2500–2850 A.U. striking vertically. Under similar conditions a film 0·11 mm. thick transmits not more than 5 per cent. of the radiations. High intensities of ultra-violet radiations and a certain degree of turbulence of the flowing film of milk increase the efficiency of activation.

An automatic recording ultra-violet meter is described which is believed to permit, when conditions and methods are standardized, of more reliable and constant control of the vitamin D potency of the irradiated milk than sporadic tests on rats (117). The activatable constituents of the milk are not confined to the butterfat. Skim milk as well as lactalbumin which carries some 18 per cent. of the total cholesterol of milk can be activated to a marked degree (112). The work of Haman & Steenbock (118) should, however, be consulted in this respect.

The physiology of the transfer of vitamin D from feed to milk, in cows receiving irradiated yeast, has been further studied by Light *et al.* (119). It was found that the

absorption of the vitamin begins between 1 and 2 hours after feeding, and that practically the whole amount fed appears in the blood. Part of it is re-excreted into the intestinal tract and found in the faeces, but the larger part is destroyed. The storage in the body appears to be much less marked than that of vitamin A.

Schoenleber & Steenbock (120) fed for 10 months to rats as their sole diet, milks fortified with vitamin D by the various methods above described, and found no injurious effects in spite of the very large intake of vitamin D.

It is interesting to note that while the feeding of irradiated yeast to cows is carried out commercially on a very large scale in the United States, two tests carried out in Germany (121, 122) with a commercial preparation of irradiated yeast have yielded unsatisfactory results, doubtless because of the unsuitable character of the yeast, of which in one case 0·8 g. daily were insufficient to cure rickets in rats completely.

The feeding of irradiated molds to cows has been studied by Thomas & Cannon (123).

Kon & Henry (124) fed to cows 2 lb. daily of cacao shell, a by-product of chocolate manufacture, and raised the vitamin D content of the milk from the winter to the summer level. The antirachitic activity of the shell is due to the action of tropical sunlight during the drying of the fermented cacao pods (Knapp & Coward (125)).

Investigations showing that when equal numbers of rat units are fed to cows, irradiated ergosterol is less efficient than irradiated yeast, have been reported in the previous review (1). More recent work of Krauss *et al.* (126) confirms this view, but the efficiency ratio is narrowed from 3 : 1 to 3 : 2. On the other hand, Russell *et al.* (127) found no difference in the efficiency of irradiated ergosterol and irradiated yeast when fed at a level of 60,000 Steenbock units (160,000 I.U.) per cow daily. At three times this level irradiated yeast was slightly more efficient. The addition of an antioxidant (hydroquinone) increased the efficiency of irradiated ergosterol at the lower level to 1·5 times that of irradiated yeast.

Using chicks Bethke *et al.* (128) have compared the antirachitic efficiencies of "yeast milk" and of irradiated milk. They found that when rat units are equalized irradiated milk is as good as cod-liver oil and more than 10 times as potent as "yeast milk". Identical results have been reported by Haman & Steenbock (118).

The almost universal belief that ergosterol is the provitamin D of cholesterol has recently been challenged by Waddell (129), who showed in carefully controlled experiments that when equal numbers of rat units are fed to chicks, irradiated cholesterol is much more potent than irradiated ergosterol and equal to cod-liver oil. His findings have been confirmed by Haman & Steenbock (118). This would go far to explain the difference in efficiency between yeast milk and irradiated milk, but, as will be shown in the section dealing with human nutrition, the explanation is totally inadequate to account for the recent clinical findings. It must be said that though much work has been done the problem of the relative activities of various forms of the antirachitic factor is still as baffling as ever.

## C. *Milk products*

### (*a*) *Cheese.*

(i) *General.* In the manufacture of cheese losses of nutritionally valuable substances may be expected to take place in the separation of the whey and in the course of ripening. Skim milk cheese is, from the start, deprived of a large part of the energy and vitamins contained in the fat of milk. On the other hand, cheese contains in a concentrated form such valuable constituents of milk as the high-quality proteins, large amounts of Ca and, in the case of whole milk cheese, up to some 35 per cent. of fat, that it is not necessary to stress its importance in nutrition.

It is not surprising that the literature dealing with nutritional aspects of cheese is increasing rapidly, and that special attention is paid to these problems in important cheese-producing countries such as Italy and America.

A review of the nutritive value of cheese and a good bibliography especially of Italian work is given by Visco (130). Martin (131) gives a popular review. Bertarelli (132) discusses the dietetic properties of cheese.

(ii) *Vitamins.* The vitamin A content of Puerto Rico native cheese has been studied biologically by Cook & Axtmayer (133). Similar studies on New Zealand cheese are reported by Frengley & Herrick (134), who state that cheese was more potent than butter when compared on the fat basis. Coward & Morgan (14) give a value of 55 I.U. per gram for English Cheddar cheese.

Allegri (135) has tested on pigeons the vitamin B value of five Italian cheeses. Vitamin $B_1$ tests on New Zealand cheese will be found in paper (134). Mameli & Cultrera (136) have compared the vitamin B content of milk with that of certain cheeses, curd, and whey, and noted some loss in the curd and a marked loss in the cheese as compared with milk. They have also compared milk with curd prepared by means of six different rennets and report the superiority of milk but no appreciable difference within the curds (137).

Estimations of the vitamin C content of various cheese are given in papers (135, 136, 137). The results are negative.

According to Mameli & Cultrera (136, 137) the vitamin D content of the Italian cheese studied by them is definitely higher than that of the corresponding amount of milk on the solid basis. It is a matter for conjecture whether their unorthodox technique measured vitamin D or some other effect. The vitamin D content of New Zealand cheese measured on the extracted fat was found to show seasonal variations (134).

(iii) *Other factors.* The biological value of the nitrogen of cheese has been studied by Frengley & Herrick (134) and by Beadles *et al.* (138). The former found by the method of Osborne, Mendel and Ferry and by that of Mitchell, that the biological value of the nitrogen of New Zealand cheese was higher than that of casein. The latter compared American Cheddar, Roquefort, Swiss cheese and Limburger with rennet milk curd, and found that the digestibility of the protein of the cheeses was slightly lowered, and that the biological value (Mitchell) of the nitrogen of Swiss cheese was unchanged, but was depressed for Limburger cheese by about 6 per cent. Apparently the processes of ripening of Limburger cheese had caused a partial destruction of some amino acid other than cystine.

The Ca and P content of various cheese is given by McCammon *et al.* (139).

(*b*) *Dried and evaporated milk.*

The partial or almost complete removal of water from liquid milk which takes place during the manufacture of dried and evaporated milks is effected by the action of heat, and is generally accompanied by exposure of the milk to the action of atmospheric oxygen at higher temperatures. The effect of these two factors on the nutritive value of the treated milks has been studied by numerous observers.

A rather optimistic review of the nutritive factors of evaporated milk is given by Rice (140).

The survival of vitamin $B_1$ in treated milks depends on the method of manufacture (141). Vitamin $B_2$ is, under the same conditions, much more heat resistant (141, 94, 142), and apparently even such drastic treatments as holding liquid milk at 170° F. for 18–24 hours before drying and even scorching of the milk powder do not affect the level of this factor in the finished product (94). It is not surprising that drying by the Merrell-Soule spray process, the Gray-Jensen spray process, or the "open" roller

method was without effect on the original vitamin B content of the liquid milk(94). The tests were carried out on chicks. It was found (also on chicks) that the vitamin $B_2$ content of dried whey was about the same as that of dried skim milk when calculated on the dry basis(143). Irradiation of evaporated milk is said to be more economical than evaporation of irradiated milk(144), though, owing to the thickness and density of films of evaporated milk, irradiation under similar conditions gives a product of lower vitamin D potency than when liquid milk is used(145).

Fairbanks & Mitchell(84) have carried out careful and extensive tests on the effect of heat on the nutritive value of milk proteins in the following skim-milk powders in comparison with liquid skim milk: (A) Roller dried: (1) low temperature, (2) normal, (3) slightly scorched, (4) markedly scorched; (B) Spray dried: (5) not pre-heated, (6) pre-heated. It was found that the true digestibility of (6) was unchanged, that of the rest was lowered slightly, while (4) had suffered appreciably. The biological value of the nitrogen of (1) and (5) was only slightly decreased (89 and 88 respectively as against 90 for the liquid milk). It fell to 82 for (2) and (6), and (3) and (4) showed a marked drop to 68 and 70 respectively. It was found that the smaller losses were due to an impairment of the cystine content of the milk, but that also, in the scorched powders, lysine was affected, and it is pointed out that the protein of these milks could not be expected to supplement cereal proteins. London and his associates(146) have compared on dogs with intestinal fistulae the nutritional value of milk powder (1) with that of raw milk (2) and found that the proteins of (1) were digested as completely as, though more slowly, than those of (2). On normal dogs the digestibility of the proteins of (1) was found to be 94·6 per cent. against 96 per cent. for (2). According to Louder & Smith(147) evaporated milk may be kept in a frozen condition for nearly 6 months without effect on its nutritive value as a sole diet for rats. Various samples of evaporated milk collected from different parts of the United States of America were found to have gained enough Cu in the process of manufacture to alleviate nutritional anaemia in rats when supplemented with chemically pure Fe(148).

Newlander & Jones(149) have compared in feeding trials on suckling pigs, evaporated milk, whole-milk powder, milk remade from unsalted butter and skim milk, and have found that raw milk was superior to evaporated milk, remade milk, and to one brand of powdered milk, but that another brand produced more economical gains in weight than the raw milk.

## II. Nutritional value of milk for human beings

### A. *Raw milk*

(*a*) *General.*

It is now generally recognized that the nutritional state of the majority of children and adolescents is far from optimal, and that no single foodstuff is so eminently qualified to raise their dietary level as milk. The same is probably true in the case of adults. In this country weighty support has been given to this opinion by the recent utterances of such bodies as the British Medical Association's Committee on Nutrition(150) and the Advisory Committee on Nutrition of the Ministry of Health, and the Department of Health for Scotland(151). The same point of view has quite recently been expressed by the Health Organization of the League of Nations(151*a*). Two notable developments, the extension of the Milk in Schools scheme(152) and the initiation of a large-scale investigation of the nutritive value of milk(153) have recently taken place in this country. The latter study, which is now in progress, consists of a comparison of 2000 children receiving no additional milk with three groups of 2000 children each, which will be given respectively one-

third and two-thirds of a pint of pasteurized milk and two-thirds of a pint of raw milk daily. Experiments on laboratory animals are also being carried out in two research Institutes.

A low intake of milk is generally associated with poverty, and reports on the relation of the state of nutrition to the intake of milk in distressed areas in England and Wales (154, 155) form instructive reading.

On the other side of the scale might be put the observations of Rosamond (156), according to which a quart of milk daily may be too much for small children. He noticed that from over 5000 children examined in his office for various ailments 6·6 per cent. benefited when their milk intake was curtailed. A certain degree of anaemia was found in all these cases.

That this amount of milk is certainly not too much for vigorous schoolboys is evident from the experience of Sibly (157) in whose school one "house", composed exclusively of lacto-vegetarians, did very well in school sports against larger "houses" of meat eaters. In comparing results the difference between the American quart and the Imperial one should be borne in mind.

Many interesting points with regard to the role of milk in infant nutrition will be found in an extensive survey by Friedman (158), and readers are referred to his paper for information concerning the feeding value of various modified milks and milk formulae which fall outside the scope of the present review. The significance of milk in adult nutrition has been discussed by Andross (159).

## B. *Treated milk*

(*a*) *Heated milk.*

(i) *General.* The nutritional significance of pasteurization is still *sub judice*. It is to be hoped that the new large-scale experiment now carried out under the aegis of the Milk Marketing Board (153) is free from the errors which made some aspects of the Lanarkshire experiment (discussed in the previous review) difficult to interpret. Ogilvie & Peden (160) have studied the gastric digestion of raw and boiled milk in seventeen infants aged from 3 to 33 months. For purposes of analyses the gastric contents were removed before, and at half-hourly intervals after test feeds with both milks. No significant differences were found.

Scurvy has been observed in a child fed for 2½ years on boiled milk (161), but a rapid cure of infantile scurvy by the administration of 1 pint of stassanized milk has been reported (162).

The case for raw milk is again stated by Sprawson (163), but views diametrically opposed to his are expressed by Soper (164).

A report (165) from the United States of America stresses the beneficial results of supplementary mid-morning feeding noted in experiments on some 760 rural school children. Pasteurized or evaporated milks were found superior in this respect to an addition of tomato concentrate.

Corry Mann (166) in experiments on himself has noticed the subjective disadvantages of omitting from his diet one-half (10 oz.) of its customary milk (pasteurized), while all the meat (containing about the same amount of protein) could be left out with impunity.

(ii) *Minerals.* Careful metabolic experiments by Daniels and her associates (167, 168) on the Ca and P requirements of children between 3 and 5 years show that these can be satisfied by 1 pint of pasteurized milk daily. The milk was added to a diet which was believed to be optimal in other respects, supplied 23 per cent. of Ca from other sources, and contained ample supplies of vitamin D. The authors point out that their experiments do not confirm the classic postulate of Sherman, that children of these ages must take 1 quart of milk daily to cover their Ca requirements.

Stearns & Jeans (169) have studied the retention of Ca and P by children (4–12 years) who derived these minerals either from milk (it is not stated whether the milk was raw or heated) or from various Ca and P salts, or from a combination of both. Of the salts the Ca phosphates gave the best retentions which were approximately equal to those from equivalent quantities of milk.

(*b*) *Irradiated, vitaminized and mineralized milk.*

Marked differences in the clinical effectiveness of various milks fortified with vitamin D and other antirachitic agents of apparently the same rat unitage have been reported in the previous review (1). Since then many new papers have been published, and it is a matter of satisfaction that much greater stress has been laid on exact experimental conditions than has so far been the rule. As a result, some misunderstandings seem to be clearing up.

Of critical papers and reviews, that of McCollum (170) deserves special mention. He explains very clearly the confusion prevalent in the United States of America in expressing the relative potencies of various antirachitics in terms of cod-liver oil, and points out the errors thus introduced into various papers. A very good critical review of the literature on the relative clinical value of various fortified milks up to the end of 1934 has been written by Bunker & Harris (171). Eliot & Powers (172) have presented a carefully drawn plan for obtaining trustworthy clinical information on the relative value of antirachitic milks. Further reviews of the literature will be found in (173, 174, 110).

For the sake of convenience all vitamin D values given in the following pages are recalculated in international units on the basis 1 Steenbock unit = 2·7 I.U.[1]

In two very carefully conducted curative experiments on "hospitalized" infants (175, 176) no difference was found between the antirachitic action of irradiated milk and of milk produced by cows given irradiated yeast when both milks contained the same number of rat units. The milks supplied some 110 I.U. per day in the first case and from 110 to 160 in the other. The results are not in accord with the statements of Hess, discussed in the previous review (1), but there seems to be little doubt that the experiments were carried out under conditions allowing greater precision. The cumulative evidence of several other studies shows that paediatricians are at present less ready than they were to suggest wide differences in the response of the infant to equal numbers of rat units from different sources. Thus (175) presents evidence that cod-liver oil is probably as effective as irradiated milk. Barnes (177) has shown that 135 I.U. daily of Zucker's cod-liver oil concentrate in milk is sufficient to protect completely a group of thirty-two normal infants from rickets, while fourteen out of twenty-five controls developed the disease. The same unitage also caused progressive healing in cases of mild rickets. Drake *et al.* (178), in comparing cod-liver oil, irradiated ergosterol, and irradiated milk, found, like Hess & Lewis, cited in (1), that 94·5 I.U. of vitamin D in the form of irradiated milk were as efficacious as 2160 I.U. of irradiated ergosterol. But surprisingly enough they also found that 270 units of ergosterol were just as good as 2160. Whether a further reduction in dosage of the irradiated ergosterol would bring it to parity with irradiated milk is not known. The lack of proportionality between dose and effect in this work is striking, as one teaspoonful of cod-liver oil was found to be at least as good as three, and the healing was by no means complete. Comments on this paper will be found in (179).

Jeans & Stearns (180) have studied the Ca retention of infants receiving from 59·4 to 135 I.U., either as irradiated evaporated milk or as milk containing Zucker's

[1] This is a generally accepted value, though Steenbock in a recent publication (118 *b*) favours a conversion factor of $3\frac{1}{3}$.

concentrate or, finally, as cod-liver oil given separately in addition to evaporated milk. No differences were found between the three groups, but the retentions were lower than those obtained in a previous study when 337·5 units were given daily.

While the bulk of the evidence points to less difference between various antirachitics than has hitherto been believed, some authors note definite variations. The results of Lewis (181) are of great interest in this respect. He found, in a curative study, mostly on negro children, that irradiated ergosterol dissolved in 24 oz. of milk was definitely more active than the same amount dissolved in oil (121·5 units of the former giving better healing than 243 of the latter). Apparently the better utilization of the irradiated ergosterol in milk was not due to increased absorption in the intestinal tract. Laboratory support to these clinical findings is given by Supplee (182), who thinks that lactalbumin may play a role. The suggestion of de Sanctis & Craig (183), that the action of vitamin D is increased in the presence of vitamin A, might also be considered in this connexion.

Passing now from comparisons of activities to clinical reports on the efficacy of different antirachitic milks, the following recent observations are of interest. A number of tests have been carried out with milk containing the Zucker cod-liver oil concentrate. Infants receiving such milk containing 400 I.U. per quart (reconstituted from evaporated milk) showed favourable progress (184). The tests were carried out by the calcium retention method, and the intakes were from 245 to 400 units daily.

McBeath (185) gave Zucker's concentrate in evaporated or fluid milk to boys and girls between 8 and 14 years, and brought about a statistically significant arrest in the development of dental caries.

A cod-liver oil concentrate, containing both vitamin A and D and incorporated in evaporated whole milk, was given by Peterman & Epstein (186) to a group of twenty-six infants. Doses of 228–485 I.U. of vitamin D and about 1100–2400 I.U. of vitamin A were found to give adequate protection.

Irradiated evaporated milk (125 I.U. per can of 14·5 oz.) when tested on "hospitalized" negro infants was shown to give good protection, but to be insufficient as a curative agent in doses of from 88 to 129 I.U. daily (187). Irradiated evaporated milk of a similar potency (10·3 I.U. per oz.) was found in another investigation to afford almost complete protection to a group of twenty-two children each of whom consumed from 71·8 to 159 I.U. daily (188). Lewis (189) was unable to confirm the previous experience of Bunker *et al.*, cited in (1) that the feeding of vitamin D milk to lactating mothers may be of value to the infant. He found that this procedure does not necessarily protect the infant from rickets.

Replies to a questionnaire on the importance of irradiated milk in the prophylaxis of rickets will be found in (190). The opinion of the New York Academy of Medicine on the value of various milks, supplemented with vitamin D, will be found in (191). The requirements of premature infants for vitamin D are notoriously high, and it is not surprising that "yeast milk" containing 432 I.U. per quart cannot afford them complete protection (173).

The latest experience in the clinical value of directly irradiated milk and of milk produced by cows fed irradiated yeast, while simplifying the problem as far as the human infant is concerned, makes that of the relative values of various antirachitics for different species still more complicated. It does not fit in with the findings of Waddell (129), discussed in a previous section of this review, and makes it clear that tests on chicken are an even less reliable guide to clinical values than tests on rats.

The bulk of the papers reviewed show that the antirachitic factor present in different forms of milk and also in cod-liver oil is effective at a surprisingly low rat dosage. The recent results of Compère *et al.* (192), who found it necessary to administer

some 2000 I.U. of vitamin D in the form of cod-liver oil and almost 7000 I.U. in the form of irradiated yeast to bring about a satisfactory cure of rickets, may be cited to show the degree of uncertainty still attaching to clinical tests.

A few papers have appeared on the problem of the iodine content of milk. Weston (193), after analysing numerous samples of dried milk collected from different parts of the United States of America, selected those with the highest iodine content, and in collaboration with a group of paediatricians carried out experiments in goitrous regions. No details are given of the results, but it is stated that milk of naturally high iodine content offers the solution of the goitre problem in children. This paper has brought in its wake the usual extravagant claims. Makers of an organic iodine compound state that they have been licensed in Wisconsin to add the preparation to milk, and the reader is informed that inorganic iodine compounds are drugs, but that iodine in organic combination is a food (194).

(*c*) *Soft-curd milk* (*artificial*).

Soft-curd milk (produced by the base exchange method, p. 197) caused higher absolute and percentage retentions of Ca when compared with whole boiled milk. The soft-curd milk contained some 20 per cent. less Ca than normal milk (195). Favourable results in feeding infants with soft-curd milk produced by the same method are reported by Rogers *et al.* (196).

## C. *Milk products*

(*a*) *Cheese.*

Mallon *et al.* (197) have found in short metabolic experiments on two healthy young women that the Ca of American Cheddar cheese was as well utilized as that of pasteurized milk. The dietetic importance of cheese is discussed by Bertarelli (132).

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